BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

```
?show files;ds;t/3,k/all
File   5:Biosis Previews(R) 1969-2002/Dec W5
         (c) 2002 BIOSIS
File   6:NTIS 1964-2003/Jan W1
         (c) 2003 NTIS, Intl Cpyrght All Rights Res
File   8:Ei Compendex(R) 1970-2003/Dec W5
         (c) 2003 Elsevier Eng.  Info. Inc.
File  34:SciSearch(R) Cited Ref Sci 1990-2002/Dec W5
         (c) 2002 Inst for Sci Info
File  65:Inside Conferences 1993-2003/Jan W1
         (c) 2003 BLDSC all rts. reserv.
File  71:ELSEVIER BIOBASE 1994-2003/Jan W1
         (c) 2003  Elsevier Science B.V.
File  73:EMBASE 1974-2003/Dec W5
         (c) 2003 Elsevier Science B.V.
File  94:JICST-EPlus 1985-2003/Oct W4
         (c)2003 Japan Science and Tech Corp(JST)
File  98:General Sci Abs/Full-Text 1984-2002/Nov
         (c) 2002 The HW Wilson Co.
File  99:Wilson Appl. Sci & Tech Abs 1983-2002/Nov
         (c) 2002 The HW Wilson Co.
File 135:NewsRx Weekly Reports 1995-2003/Dec W5
         (c) 2003 NewsRx
File 143:Biol. & Agric. Index 1983-2002/Nov
         (c) 2002 The HW Wilson Co
File 144:Pascal 1973-2002/Dec W4
         (c) 2002 INIST/CNRS
File 155:MEDLINE(R) 1966-2002/Dec W3
File 172:EMBASE Alert 2003/Jan W1
         (c) 2003 Elsevier Science B.V.
File 266:FEDRIP 2002/Nov
         Comp & dist by NTIS, Intl Copyright All Rights Res
File 315:ChemEng & Biotec Abs 1970-2002/Nov
         (c) 2002 DECHEMA
File 357:Derwent Biotech Res. _1982-2003/Dec W5
         (c) 2003 Thomson Derwent & ISI
File 358:Current BioTech Abs 1983-2002/Nov
          (c) 2002 DECHEMA
File 369:New Scientist 1994-2003/Dec W4
         (c) 2003 Reed Business Information Ltd.
File 370:Science 1996-1999/Jul W3
         (c) 1999 AAAS
File 399:CA SEARCH(R) 1967-2003/UD=13802
         (c) 2003 American Chemical Society
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
         (c) 1998 Inst for Sci Info
File  40:Enviroline(R) 1975-2002/Dec
File  50:CAB Abstracts 1972-2002/Nov
         (c) 2002 CAB International
File  68:Env.Bib. 1972-2002/Jun
         (c) 2002 Internl Academy at Santa Barbara
File 103:Energy SciTec 1974-2002/Dec B2
         (c) 2002 Contains copyrighted material
File 156:ToxFile 1965-2002/Nov W3
         (c) format only 2002 The Dialog Corporation
File 162:CAB Health 1983-2002/Nov
         (c) 2002 CAB International
File 305:Analytical Abstracts 1980-2002/Dec W2
         (c) 2002 Royal Soc Chemistry
File  35:Dissertation Abs Online 1861-2003/Dec
         (c) 2003 ProQuest Info&Learning
File  48:SPORTDiscus 1962-2002/Dec
         (c) 2002 Sport Information Resource Centre
File  91:MANTIS(TM) 1880-2002/Oct
         2002 (c) Action Potential
File 149:TGG Health&Wellness DB(SM) 1976-2003/Dec W4
         (c) 2003 The Gale Group
```

```
File 159:Cancerlit 1975-2002/Oct
         (c) format only 2002 Dialog Corporation
File 164:Allied & Complementary Medicine 1984-2002/Dec
          (c) 2002 BLHCIS
File 442:AMA  Journals 1982-2003/Jan B2
         (c)2003 Amer Med Assn -FARS/DARS apply
File 444:New England Journal of Med. 1985-2003/Jan W1
         (c) 2003 Mass. Med. Soc.
File 467:ExtraMED(tm) 2000/Dec
         (c) 2001 Informania Ltd.

Set     Items   Description
S1          0   CPG (S) OLIGONUCLEOTIDE? (S) 3' (S) (MODIF? OR SUBSTITUT?)
S2         35   CPG (S) (MODIF? OR SUBSTITUT?) (S) (HALO? OR ALKOXY OR ALK-
               YL)
S3         19   RD (unique items)
S4          0   SHOW FILES
S5         16   S2 AND OLIGO?
S6         12   RD (unique items)
S7          8   S2 AND IMMUN?
S8          3   RD (unique items)
S9          0   (OLIGONUCLEOTIDE? OR POLYNUCLEOTIDE?) (S) (3' (4N) (SUBSTI-
               TUT? OR MODIF?))
S10      1905   (OLIGONUCLEOTIDE? OR POLYNUCLEOTIDE?) AND (3 (4N) MODIF?)
S11       842   (OLIGONUCLEOTIDE? OR POLYNUCLEOTIDE?) AND (3 (4N) SUBSTITU-
               T?)
S12       161   (S19 OR S10) AND (ALKYL? OR HALO? OR ALKOXY?)
S13       106   RD (unique items)
S14         2   S13 AND (?CG? OR ?CPG?)
S15        14   S13 AND IMMUN?
>>>KWIC option is not available in file(s): 399
```

```
Set     Items   Description
S1          0   CPG (S) OLIGONUCLEOTIDE? (S) 3' (S) (MODIF? OR SUBSTITUT?)
S2         35   CPG (S) (MODIF? OR SUBSTITUT?) (S) (HALO? OR ALKOXY OR ALK-
              YL)
S3         19   RD (unique items)
S4          0   SHOW FILES
S5         16   S2 AND OLIGO?
S6         12   RD (unique items)
>>>KWIC option is not available in file(s): 399
```

6/3,K/1 (Item 1 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

11012739 Genuine Article#: 596BL No. References: 37

Title: Design, synthesis, and immunostimulatory properties of *CpG* DNAs containing *alkyl*-linker *substitutions*: Role of nucleosides in the flanking sequences

Author(s): Yu D; Kandimalla ER; Cong YP; Tang J; Tang JY; Zhao QY; Agrawal S (REPRINT)
Corporate Source: Hybridon Inc,345 Vassar St/Cambridge//MA/02139 (REPRINT); Hybridon Inc,Cambridge//MA/02139

Journal: JOURNAL OF MEDICINAL CHEMISTRY, 2002, V45, N20 (SEP 26), P 4540-4548
ISSN: 0022-2623 Publication date: 20020926
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

Title: Design, synthesis, and immunostimulatory properties of *CpG* DNAs containing *alkyl*-linker *substitutions*: Role of nucleosides in the flanking sequences

Abstract: Bacterial and synthetic DNA containing unmethylated *CpG* dinucleotides activate the innate immune system and promote Th1-like immune responses. Recently, a receptor, TLR9, has been shown to recognize *CpG* DNA and activate immune cascade. But there have been no reports on the molecular mechanisms of recognition between *CpG* DNA and the receptor(s). Our earlier studies described a number of the chemical and structural characteristics of *CpG* dinucleotide and the sequences flanking the *CpG* dinucleotide that are critical for immunostimulatory activity. In the present study, we examined the effect of the presence and absence of a nucleoside in the flanking sequences by replacing one or two natural deoxyribonucleosides at various positions with one or more *alkyl*- (C2-C12), branched *alkyl*-(glyceryl or aminobutyrylpropanediol), or ethyleneglycol- (tri or hexa) linkers. The results suggest that a linker *substitution* at the first two nucleoside positions adjacent to the *CpG* dinucleotide on the 5'- or the 3'-side neutralizes the immunostimulatory activity, as determined by in vitro mouse spleen cell proliferation, cytokine secretion, and in vivo mouse spleen enlargement. The same *substitutions* placed about three to six nucleotides away from the *CpG* dinucleotide either did not affect or potentiated immunostimulatory activity compared with parent *CpG*-DNA without *modifications*. *Substitution* of deoxyribonucleosides with a C3 or C4 *alkyl*-linker was found to be optimal for potentiating immunostimulatory activity.

...Identifiers--CAPPED *OLIGODEOXYNUCLEOTIDE* PHOSPHOROTHIOATES; MYCOBACTERIUM-BOVIS BCG; CHEMICAL MODIFICATIONS; BACTERIAL-DNA; *OLIGONUCLEOTIDES; *MICE; STABILITY; PHARMACOKINETICS; STIMULATION; PROTECTION

6/3,K/2 (Item 2 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02166816 Genuine Article#: KF932 No. References: 21

Title: PHOTOCHEMICAL CLEAVAGE OF *OLIGONUCLEOTIDES* FROM SOLID-PHASE

SUPPORTS
Author(s): GREENBERG MM
Corporate Source: COLORADO STATE UNIV,DEPT CHEM/FT COLLINS//CO/80523
Journal: TETRAHEDRON LETTERS, 1993, V34, N2 (JAN 8), P251-254
ISSN: 0040-4039
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

Title: PHOTOCHEMICAL CLEAVAGE OF *OLIGONUCLEOTIDES* FROM SOLID-PHASE SUPPORTS
Abstract: 5'-O-Dimethoxytrityl-3'-O-succinatothymidine is covalently, linked to long chain *alkyl* amine controllled pore glass support (LCAA-*CPG*) via an o-nitrobenzyl group. The *modified* solid phase synthesis support is compatible with standard automated phosphoramidite based *oligonucleotide* synthesis. Cleavage from the solid support is achieved via CuSO4 filtered photolysis. Isolated yields are comparable with those obtained from other *oligonucleotide* supports.

6/3,K/3 (Item 1 from file: 135)
DIALOG(R)File 135:NewsRx Weekly Reports

0000071892 (USE FORMAT 7 OR 9 FOR FULLTEXT)
Enhanced immunomodulatory activity described
Biotech Week, December 25, 2002, p.63

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English
RECORD TYPE: FULLTEXT
WORD COUNT: 487

...TEXT: CpG DNA molecules linked through their 3' ends (immunomers), as demonstrated in cell culture and murine models.

The report provides insights into designing potent immunomodulatory *oligonucleotides* (IMO) for specific immunotherapeutic applications. Hybridon recently described in other publications that linking *CpG* DNA through the 5' end abrogates immunostimulatory activity, and that adding *alkyl*-linkers in *substitution* of certain nucleotides in the 5' flanking region - but not the 3' flanking region - increases immunostimulatory activity, also as demonstrated in cell culture or murine...
... in a predictable fashion.

"We continue to add building blocks to our understanding of the structure/activity relationship and the design of our proprietary immunomodulatory *oligonucleotides*," said Sudhir Agrawal, Hybridon's chief scientific officer. "We believe that this knowledge will allow us to design and develop potent molecules with specific immune...

...will be the preferred partner in this field."

The study, published in the journal Nucleic Acids Research and entitled "'Immunomers' - novel 3'-3' - linked CpG *oligodeoxyribonucleotides* as potent immunomodulatory agents," reports that, in cell cultures and murine models, CpG DNA molecules, when linked through their 3' ends, showed enhanced activity as...

6/3,K/4 (Item 2 from file: 135)
DIALOG(R)File 135:NewsRx Weekly Reports

0000069544 (USE FORMAT 7 OR 9 FOR FULLTEXT)
Interaction of novel DNA structures and immune system activation studied
Biotech Week, November 13, 2002, p.54

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English
RECORD TYPE: FULLTEXT
WORD COUNT: 380

...TEXT: activation of the immune system.

The articles, recently published in the Journal of Medicinal Chemistry and in Bioconjugate Chemistry , provide insights into designing potent immunomodulatory *oligonucleotides* (IMOs) for specific immunotherapeutic applications, the company says.

... of the on-going effort at Hybridon to develop a thorough understanding of the structure/activity relationships and to create an inventory of proprietary immunomodulatory *oligonucleotide* compounds to effectively treat patients."

The publication in the Journal of Medicinal Chemistry (Design, synthesis, and immunostimulatory properties of *CpG* DNAs containing *alkyl*-linker *substitutions*: Role of nucleosides in the flanking sequences. J Med Chem, 2002;45(20):4540-4548), reports that in murine cell culture experiments the *substitution* of certain nucleotides with *alkyl* -linkers in the 5'-flanking sequence to the *CpG* dinucleotide increased immunostimulatory activity, whereas the same *substitution* on the 3'-flanking sequence did not affect activity compared with the parent *CpG* DNA. In this study, the increase in immunostimulatory activity resulted from the structural *modifications* introduced in the *CpG* DNA.

The Bioconjugate Chemistry (Conjugation of ligands at the 5'-end of CpG DNA affects immunostimulatory activity. Bioconjug Chem, 2002;13(5):966-974) publication...

6/3,K/5 (Item 1 from file: 315)

DIALOG(R)File 315:ChemEng & Biotec Abs

273838 CEABA Accession No.: 22-05-004844 DOCUMENT TYPE: Journal

Title: Universal solid supports for the synthesis of *oligonucleotides* with terminal 3'-phosphates

CORPORATE SOURCE: Polish Acad. of Sciences Posnan Poland
JOURNAL: Nucleic Acids Research, Volume: 17, Issue: 18, Page(s): 7149-7158
CODEN: NARHAD ISSN: 03051048
PUBLICATION DATE: 1989 (890000) LANGUAGE: English

Title: Universal solid supports for the synthesis of *oligonucleotides* with terminal 3'-phosphates

ABSTRACT: Two supports based on controlled pore glass (*CPG*) are prepared by *modifying* *CPG* with: (1) methacrylic acid derivatives and 2-mercaptoethanol, (2) *aminoalkylsilane* , succinic anhydride and benzidine. Both supports are usable for phosphoramidite chemistry and probably also for phosphorotriester and H-phosphanate approaches. (Cohnen)

6/3,K/6 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

0296334 DBR Accession No.: 2002-18181 PATENT

Novel *oligonucleotide* chimera to selectively prevent gene expression in a sequence-specific manner, comprising a chimera of modified arabinose and 2'-deoxy sugars hybridizing to a single stranded RNA - antisense *oligonucleotide* use in gene therapy and gene expression control

AUTHOR: DAMHA M J; PARNIAK M A; LOK C; VIAZOVKINA E
PATENT ASSIGNEE: UNIV MCGILL 2002
PATENT NUMBER: WO 200220773 PATENT DATE: 20020314 WPI ACCESSION NO.: 2002-499783 (200253)
PRIORITY APPLIC. NO.: US 230414 APPLIC. DATE: 20000906
NATIONAL APPLIC. NO.: WO 2001CA1252 APPLIC. DATE: 20010904
LANGUAGE: English

Novel *oligonucleotide* chimera to selectively prevent gene expression in a sequence-specific manner, comprising a chimera of modified arabinose and 2'-deoxy sugars hybridizing to a single stranded RNA - antisense *oligonucleotide* use in gene therapy and gene expression control

ABSTRACT: DERWENT ABSTRACT: NOVELTY - An *oligonucleotide* chimera (I) to selectively prevent gene expression in a sequence-specific manner, comprising a chimera of *modified* arabinose and 2'-deoxy sugars hybridizing to a single stranded RNA, is new. DETAILED DESCRIPTION - An *oligonucleotide* chimera (I) to selectively prevent gene expression in a sequence-specific manner, comprising a chimera of *modified* arabinose and 2'-deoxy sugars hybridizing to a single stranded RNA, is new. (I) hybridizes to a single stranded RNA to induce nuclease stability, binding strength of hybridization to complementary RNA sequences, permeability of the *oligonucleotide* into cells, cleavage of target RNA by RNaseH, or physical blockage of ribose translocation (translation arrest). An INDEPENDENT CLAIM is also included for a pharmaceutical...

... sequence-specific manner, comprising (I). ACTIVITY - None given. MECHANISM OF ACTION - Antisense gene therapy; inhibitor of translation of single-stranded RNA. The ability of antisense *oligonucleotides* constructed from 2'-deoxy-2'-fluoro-beta-D-arabinonucleotides (FANA) flanking a series of 2'-deoxyribose nucleotide residues of variable length (S-FANA gapmer) to...

... stably transfected with the luciferase gene, was determined. The efficacy of S-FANA gapmer to inhibit intracellular luciferase expression was compared with identical sequence antisense *oligonucleotides* constructed entirely from FANA or entire from 2'-deoxyribonucleotides. The specific antisense *oligonucleotide* sequences were 5'-ATATCCTTGTCGTATCCC-3', (complementary to bases 1511-1528 of the coding region of the luciferase gene). As a control randomized *oligonucleotide* sequences (5'-TAATCCCTATCGTCGCTT-3') were used. The ability of *oligonucleotides* complementary to a specific region of mRNA coding for luciferase was tested for inhibition of luciferase activity expression in Hela Z1/5 cells. The results showing the ability of antisense *oligonucleotides* (sequence 5'-ATATCCTTGTCGTATCCC-3'), to inhibit X1/5 cell luciferase activity were represented graphically. The cells were exposed to 250 nM of antisense *oligonucleotide*, for 24 hours prior to assay of luciferase activity. The antisense *oligonucleotide* constructed entirely from beta-D-2'-deoxyribose with phosphodiester bonds (PO-DNA) was unable to effect any inhibition of X1/5 cell luciferase activity, whereas the antisense *oligonucleotide* constructed entirely from beta-D-2'-deoxyribose with phosphorothioate bonds (PS-DNA) provided 60 % inhibition. Antisense *oligonucleotides* constructed entirely from 2'-deoxy-2'-fluoro-beta-D-arabino nucleotides with either phosphodiester bonds (PO-FANA) or phosphorothioate bonds (PS-FANA) provided 55 % and...

... useful in the preparation of a probe or laboratory reagent for cleaving single stranded RNA. (All claimed). (I) is useful as a model of antisense *oligonucleotide* agents, and serves as therapeutics and/or valuable tools for studying and controlling gene expression in cells and organisms. EXAMPLE - The synthesis of 2'-deoxy...

...Synthesis of S-FANA and S-(FANA-DNA-FANA) chimeras were synthesized on a 1 micromol scale using an Expedite 8909 DNA-synthesizer. Long-chain *alkylamine* controlled-pore glass (LCAA-*CPG*) was used as the solid support. The synthesis cycle consisted of detritylation of nucleoside/tide bound to *CPG* (3 % trichloroacetic acid/dichloromethane) for 150 seconds, and coupling of 2'-F-arabinonucleoside or 2'-deoxyribonucleoside 3'-phosphoramidite monomers for 15 minutes, using 50 mg...

...2-benzodithiol-3-one in acetonitrile (10 minutes), and the solid support was dried by adding the capping reagent for 5 seconds. Following chain assembly, *oligonucleotides* were cleaved from the solid support and deprotected. The crude *oligomers* were purified by preparative gel electrophoresis (24 % acrylamide, 7 M Urea) followed by desalting (Sephadex G-25 (RTM)).(40 pages)

DESCRIPTORS: *oligonucleotide* chimera, arabinose, 2'-deoxy sug[illegible] modification, sense, antisense *oligonucleotide*, appl. gene expression control, ss RNA hybridization, cleavage, translation prevention, drug screening, DNA probe, therapy, gene therapy (21, 49)

6/3,K/7 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0296060 DBR Accession No.: 2002-17907 PATENT
Preparing peptide linked *oligomeric* compound useful for diagnostics, therapeutics and as research reagents and kits by employing equimolar amounts functionalized *oligomeric* compounds and peptide reagents - *oligonucleotide* preparation and HPLC for diagnosis and gene therapy
AUTHOR: MANOHARAN M; GUZAEV A P
PATENT ASSIGNEE: ISIS PHARM INC 2002
PATENT NUMBER: WO 200220544 PATENT DATE: 20020314 WPI ACCESSION NO.: 2002-489670 (200252)
PRIORITY APPLIC. NO.: US 658517 APPLIC. DATE: 20000908
NATIONAL APPLIC. NO.: WO 2001US28083 APPLIC. DATE: 20010907
LANGUAGE: English

Preparing peptide linked *oligomeric* compound useful for diagnostics, therapeutics and as research reagents and kits by employing equimolar amounts functionalized *oligomeric* compounds and peptide reagents - *oligonucleotide* preparation and HPLC for diagnosis and gene therapy
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Preparing peptide linked *oligomeric* compound (PC) by deprotecting protected hydroxyl group (HG) of compound derivatizing support medium, reacting deprotected HG with nucleoside, to form extended compound from which capped compound is formed, oxidized, cleaved to form *oligomeric* compound having reactive sulfur moiety (RM). RM is reacted with peptide with functional group reactive with sulfur moiety, to form PC. DETAILED DESCRIPTION - Preparing (M1...

... one nucleoside to give a further oxidized compound; (h) cleaving the oxidized compound or the further oxidized compound from the support medium to give the *oligomeric* compound comprising a linking moiety; (i) treating the linking moiety attached to the *oligomeric* compound with reagents effective to form a reactive sulfur moiety on the linking moiety; and (j) reacting the reactive sulfur moiety with a peptide which...

...reactive with the sulfur moiety thereby forming PC. PC is of the formula (I)-(III), and is prepared by the following method (M2): providing an *oligomeric* compound of the formula (IV), (V) or (VI), reacting the *oligomeric* compound with a functionalized peptide having a -SH functional group thereby forming PC. T1 = hydrogen or hydroxyl protecting group; X1 = O, Pg-O-, S, Pg-S-, C1-C10 *alkyl*, CH3(CH2)g-O-, R2R3N- or a group remaining from coupling or a chiral auxiliary; X2 = O or S; g = 0-10; Pg = CH3, -CH2CH2CN...

...nitro-2-yl-ethylbenzene, 2-yl-ethyl-methyl sulfonate, methyl-N-trifluoro acetyl ethyl, acetoxy phenoxy ethyl, or a blocking group; R2, R3 = hydrogen, C1-C10 *alkyl*, *cycloalkyl* or aryl, or optionally, R2 and R3, together with the nitrogen atom to which they are attached form a cyclic moiety; Bx = a heterocyclic base...

...C1) attached to a support medium having the formula (XIX), where R8 is a hydrogen, a hydroxyl protecting group, a nucleoside, a nucleotide or an *oligomeric* compound; Q1 is C1-C12 *alkyl*, -((CH2)mm-O-(CH2)mm')p-, aryl or alkaryl; Q3 is C1-C12 *alkyl*, aryl or alkaryl; p is 1-6; mm and mm' are 1-6; and Sm is a support medium; and (2) PC having the formula (XX), where R9 is an *oligomeric* compound, Q1, p, mm and mm' are as described above, and Q2 is C2-C12 *alkyl*. BIOTECHNOLOGY - Preferred Method: In (M1), the deprotected hydroxyl group is reacted with 2', 3' or 5'-phosphoramidite or preferably 2', 3', or

5'-H-phosphonate...

... and the functional group is a disulfide group. The support medium is derivatized with a compound comprising a protected hydroxyl group such as 3'-thiol-*modifier* C3 S-S *CPG* (DMT-O-(CH2)3-S-S-(CH2)3-O-succinyl -LCAA-*CPG*). The support medium is derivatized with the compound comprising an optionally protected hydroxyl group, has the formula (XXI), where R9 is H or hydroxyl protecting group; Q4 is C1-C12 *alkyl* , alkaryl or -((CH2)mm-O-(CH2)mm')p-; Q3, Sm, p, mm and mm' are as described above. Most preferably, Q4 is propyl and Q3...

... an internal disulfide group. The bifunctional compound has the formula H2N-(CH2)q-S-S-(CH2)q-NH2, where each q is 2-6. The *oligomeric* compound comprises 5-50 nucleosides, preferably, 15-25 nucleosides. PC produced by the above mentioned method has one of the formulas (XII), (XXIII) or (XXIV...

... is 2', 3' or 5' phosphoramidite. Preferred Compound: In (C1) and PC, Q1 is most preferably propyl, and Q3 is preferably methyl. J = C1-C12 *alkyl* or -Q1-G2-Q2-; G = -NH-C(O)-, -NH-C(O)-NH-, -NH-C(S)-NH-, -NH-O-, or -NH-C(O)-O-; X1 = R5-O-, O, Pg-O-, S, Pg-S-, C1-C10 *alkyl*, CH3(CH2)g-O-, R2R3N- or a group remaining from coupling or a chiral auxiliary; R5 = CH3, -CH2CH2CN, -C(CH3), (CH3)-...R3, Bx, R1 and n = as described above. ACTIVITY - None given. MECHANISM OF ACTION - None given. USE - (M1) is useful for preparing PC (claimed). The *oligomeric* compounds can be used in diagnostics, therapeutics and as research reagents and kits. They can also be used in pharmaceutical compositions by including a suitable...

...and chloroplasts) of eukaryotic cells) having a disease characterized by the undesired production of a protein. For this purpose, the organism is contacted with an *oligomer* having a sequence that is capable of specifically hybridizing with a strand of nucleic acid encoding the undesirable protein. ADVANTAGE - (M1) is suitable for large...

... synthesis of PC (claimed). The methods provide improved synthetic schemes which avoid the problem of prior art. The synthetic methods employed equimolar amounts of functionalized *oligomeric* compounds and peptide reagents which has successfully resulted in large scale synthesis. This scaled up synthesis is significantly larger than any synthesis method described previously. The methods are highly economical. EXAMPLE - 20-mer *oligonucleotide* phosphorothioate 2'-methoxyethoxy (MOE, 2'-O-CH2CH2-O-CH3) gapmers were synthesized on ABI 380B DNA synthesizer on a 25 mmol scale. The use of 3'-thiol-*modifier* C3 S-S *CPG* gave a 3'-O-P(=S) (-O)-(CH2)3-S-S-CH2)3-OH group at the 3'-terminus of selected *oligonucleotides* upon cleavage from the solid support. High performance liquid chromatography (HPLC) purified, 3'-disulfide *modified* *oligonucleotide* (1000 OD) was treated with dithiothreitol(DTT) for 2 hours. The reaction mixture was directly loaded onto an HPLC column. The column was eluted using...

... buffer B, and linear gradient from 0 to 45% of B in 60 minutes. The fractions containing 3'-O-P(=S) (-O)-(CH2)3-SH *modified* *oligonucleotide* were collected directly to a solution of 2,2'-dithiodipyridine in MeCN. The reaction mixture was kept for 2 hours and concentrated in vacuum. The excess of 2,2'-dithiodipyridine was extracted with ethyl acetate. The activated *oligonucleotide* having a 3'-O-P(=S) (-O)-(CH2)3-S-S-(2-pyridine) was purified by HPLC. Activated *oligonucleotide* having a sequence of AGCTTCTTTGCACATGTAAA (S2) (16518-3'-SSPy) *oligonucleotide* in HPLC buffer was diluted with 1 M KCl 4.5 g urea, 5 M NH4OAc, a solution of 0.1 M NH4OAc, 2 M...

... product, therefore 0.25 equivalent of Tat-SH peptide was further added and the reaction mixture left for 2 hours. The retention times of the *oligonucleotide*-peptide conjugate and the starting *oligonucleotide* were 14.2 and 23.5 minutes. The conjugate was purified by ion exchange HPLC on a Mono Q column, buffer A: 0.1 M...

... column. The 16518-3'-Tat conjugate was eluted at 36-44 minutes. The collected fractions were combined, evaporated in vacuo and desalted. The Tat-conjugated *oligomer* (S2), 16518-3'-Tat) obtained was more than 99% pure according to the analysis by reversed phase HPLC and ion exchange HPLC. (124 pages)
DESCRIPTORS: phosphorothioate *oligonucleotide* prep., large-scale synth., ionexchange chromatography, HPLC, appl. bacterium, yeast, protozoon, alga, plant, mammal DNA-RNA transcription, RNA-protein translation, hereditary, metabolic, cellular control disorder...

6/3,K/8 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0292411 DBR Accession No.: 2002-14258 PATENT
Compound for binding macromolecule to substrate surface or conjugation targets, contains phosphorous containing reactive group, hydrazide protecting group and benzene ring, and has predefined formula - DNA and RNA immobilization useful in DNA chip and DNA biosensor production
AUTHOR: RADDATZ S; MUELLER-IBELER J; SCHWEITZER M; BRUECHER C; WINDHAB N; HAVENS J R; ONOFREY T J; GREEF C H; WANG D
PATENT ASSIGNEE: NANOGEN INC 2002
PATENT NUMBER: WO 200214558 PATENT DATE: 20020221 WPI ACCESSION NO.: 2002-404476 (200243)
PRIORITY APPLIC. NO.: WO 200022205 APPLIC. DATE: 20000811
NATIONAL APPLIC. NO.: WO 2001US41663 APPLIC. DATE: 20010810
LANGUAGE: English

...ABSTRACT: amide linkage; Pr = phosphorus bearing reactive group chosen from (a) and (b) Ra, Rb = 1-12C hydrocarbon; Rc = 2-cyanoethyl, allyl, methyl, ethyl and other *alkyl* moieties; Pga = hydrazide protecting group; and m = 1-3. INDEPENDENT CLAIMS are also included for the following: (1) compound of formula (II), (III), (IV), (V), or (VI); (2) method of producing *modified* macromolecule which involves contacting a macromolecule to be *modified* with a compound chosen from compound of formulae (I-VI), the macromolecule has a reactive hydroxyl group and phosphorus bearing reactive group, phosphorus forms a covalent bond with oxygen of reactive hydroxyl group, thus producing *modified* macromolecule; (3) *modified* macromolecule comprising compound (I) which is covalently attached to a macromolecule through Pr group; (4) Use of *modified* macromolecule in a conjugation reaction with a second molecule in solution, where the *modified* macromolecule is processed to produce at least one reactive hydrazide moiety on the *modified* macromolecule, the second molecule comprises a moiety reactive with hydrazide; and (5) substrate comprising immobilized macromolecules, where the immobilization linkage has structure (S). (II) is...

...hydrocarbon linker moiety, optionally with 1-10 hetero atoms chosen from oxygen, nitrogen, sulfur and phosphorus, in functional linkage; Pl = (c); Rp = hydrogen, electron pair, *alkyl* or cyano *alkyl* moiety; Ma = macromolecule; n = 0 if hydrazide is attached to Rh by double bond; and n = 1 if hydrazide is attached to Rh by single...

... technology, surface plasmon resonance experiments and biosensor applications. ADVANTAGE - Higher rate of immobilization, higher stability of attachment and potential to obtain higher amounts of immobilized *oligosaccharide* onto the substrate surface in less time, are enabled. Multiple binding sites per bound entity, stability in broad pH range, capability of molecular attachment under...

... ethyl acetate/n-heptane 2/3 with trace triethylamine) to obtain 6-((2cyanoethoxy)(diisopropyl amino) phosphanyloxy)-N'-tritylhexanohydr azide (3.19) as a pale yellow foam. *Oligos* (e.g. DNA, RNA, peptide nucleic acid (PNA), etc.) were synthesized using solid phase phosphoramidite chemistry on an automated *oligo* synthesizer. The

phosphoramidite with the protected hydrazide was applied as 0.1 M solution in acetonitrile and coupled at the desired location in the sequence using standard activated reagents and coupling times. The *CPG* bound *oligo* (1 mmol) was placed in a 1.5 ml test tube and treated with 2.0 ml conc.NH4OH. After 2 hours, at 55 degreesC, the ammonia solution was removed and evaporated under reduced pressure. The trityl protected hydrazide *oligo* was purified by reverse phase high performance liquid chromatography (HPLC). The fractions containing the trityl-on product were pooled and evaporated and the trityl protecting group was removed by treating the *oligo* with 80 % acetic acid for 30 minutes at RT. The acid was removed in vacuo, and the residue was dissolved in water, then extracted twice...

... The aqueous layer was dried again and re-dissolved. Analytical HPLC showed a single product which was employed for further reactions without purification. Synthesis of *oligo* 09: Hydrazide-15 mer: (dp1a-TTTTTTTTTTTTTTT-3') involved synthesis and deprotection with amidite compound 1a. The trityl protected hydrazide *oligo* was purified by reverse phase HPLC using a Merck LiChrospher RP 18, 10 microM, column using 0.1 M triethylammonium acetate pH = 7.0 (TEAA...

6/3,K/9 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0114824 DBR Accession No.: 91-02466 PATENT
***Oligonucleotide* derivative - DNA or RNA synthesis with improved thermostability; potential application in DNA probe or RNA probe construction**
PATENT ASSIGNEE: Ajinomoto 1990
PATENT NUMBER: JP 2264792 PATENT DATE: 901029 WPI ACCESSION NO.: 90-365919 (9049)
PRIORITY APPLIC. NO.: JP 8985456 APPLIC. DATE: 890404
NATIONAL APPLIC. NO.: JP 8985456 APPLIC. DATE: 890404
LANGUAGE: Japanese

***Oligonucleotide* derivative**
ABSTRACT: New *oligonucleotide* derivatives are of formula (I) or (II), where: R is H, acryl or a phosphoryl *substituted* group; T is thymine-1-yl; Y1, Y2 and Y3 are H, hydroxyl, lower *alkyloxy* or lower *alkylsilyl* ; B is thymine-1-yl, adenine-9-yl, guanine-9-yl, cytosine-9-yl, uracil-1-yl or hypoxanthine-9-yl; n is 1-100; X is monomethoxytrityl, dimethoxytrityl or a phosphoryl *substituted* group; Y is H, o-chlorophenyl phosphoric acid, -P(OCH3)-N-(CH(CH3)2)2, -P(OCH2CH2CN)-N-(CH(CH3)2)2, or a -CO-(CH2)m-CONH-(CH2)n-*CPG* derivative (m and n being 1-100); and if X is a phosphoryl *substituted* group, Y is H. These *oligonucleotide* derivatives may be used for efficient detection of mRNA for cloning of useful genes. *Oligonucleotide* and nucleotide derivatives are both new. The thermostability (Tm value) of deca-2'-o-methyl- 5-methyluridylic acid is excellent. (10pp)
DESCRIPTORS: DNA, RNA *oligonucleotide* der. synth., nucleotide der. synth. with improved thermostability, pot. appl. DNA probe, RNA probe construction

6/3,K/10 (Item 1 from file: 370)
DIALOG(R)File 370:Science

00501178 (USE 9 FOR FULLTEXT)
Oxidative Thymine Dimer Repair in the DNA Helix
Dandliker, Peter J.; Holmlin, R. Erik; Barton, Jacqueline K.
Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA.
Science Vol. 275 5305 pp. 1465

Publication Date: 3-07-1997 (970307) Publication Year: 1997
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2426

(THIS IS THE FULLTEXT)

...Text: 3+) was found to catalyze the repair of a thymine dimer incorporated site-specifically (B9) in the center of a synthetic 16-base pair (bp) *oligonucleotide* duplex. Excitation of the rhodium complex at 400 nm in the presence of dimer-containing duplex resulted in the disappearance of the dimer-containing strand in a first-order kinetic process, with concomitant appearance of the repaired *oligonucleotide* (Fig. 1). Quantitative repair has been observed with concentrations of rhodium complex as low as 500 nM (B10) and a >10-fold excess of DNA duplex. This synthetic repair system is remarkable in that the metal complex is activated by visible light. Repair of dimer-containing *oligonucleotide* substrates can be accomplished with catalytic amounts of metal complex and sunlight...2 to 3% CH.inf(3)CN for 10 min, then 3 to 4% CH.inf(3)CN for 30 min); under these conditions, each *oligonucleotide* elutes with a characteristic retention time. The identity of the individual compounds was confirmed by coinjection with authentic samples. Electrospray ionization mass spectrometric analysis of...

...T.inf(R) = 17 min decreased steadily with increasing irradiation time, while the corresponding repaired strand (T.inf(R) = 26 min) smoothly appeared. The metallated *oligonucleotide* eluted at T.inf(R) = 45 min and is not shown here. Reaction conditions were identical to those in Fig. 1 except for the HPLCRh-modified *oligomer* and equally in the 5 (prime) -CG-3 (prime) step and 5 (prime) -GT-3 (prime) step for the 3 (prime) -Rh-modified duplex. With...

References and Notes:
...9. Thymine dimer-containing *oligonucleotides* are prepared by photodimerization of single-stranded DNA at 330 nm with acetophenone as a sensitizer. The chromatogram of the photoproducts and the product ratios ...15. Rhodium-*modified* *oligonucleotides* were prepared by coupling the complex, Rh(phi).inf(2)bpy (prime) .sup(3+) (bpy (prime) , 4-butyric acid 4 (prime) -methyl-2,2 (prime) -bipyridine), activated as the N-succinimidyl ester, to alkylamino functionalized *oligonucleotides* attached to controlled pore glass (*CPG*). The 3 (prime) -alkylamino DNA was prepared by standard phosphoramidite synthesis starting from C7-amino-link 500 angstrom *CPG* (Glen Research) and selective deprotection of the R-N(H)-Fmoc by treatment with 20% piperidine in DMF (v/v) at ambient temperature for 30 min. The 5 (prime) -alkylamino DNA was prepared from dT 2,000 angstrom *CPG* (Glen Research), followed by standard phosphoramidite synthesis and functionalization with a nonamethylene amino-*alkyl* linker [L. Wachter, J. A. Jablonski, K. L. Ramachandran, Nucleic Acids Res. 14, 7985 (1986)]. Activation of racemic metal complex, coupling to DNA, and HPLC purification of the diastereomeric (Delta) -Rh and (Lambda) -Rh conjugates was identical for 3 (prime) -and 5 (prime) -functionalized *oligonucleotides* [see (B14 ...
...strand cleavage at several orders of magnitude lower intensity than required to detect rhodium reaction near the tethered end of the duplex in Rh-modified *oligomers*. Photocleavage reactions as a function of concentration of Rh-modified duplexes also indicate that interduplex reaction is negligible at duplex concentrations of <=25 (mu) M...

6/3,K/11 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

135257422 CA: 135(18)257422m JOURNAL
Synthesis of oligodeoxyribonucleotides containing oleylamine moieties

AUTHOR(S): Andreev, S. Yu.; Antsypovich, S. I.; Volkov, E. M.; Romanova, E. A.; Hianik, T.; Oretskaya, T. S.
LOCATION: Department of Chemistry and Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia, 119899
JOURNAL: Russ. J. Bioorg. Chem. DATE: 2001 VOLUME: 27 NUMBER: 3
PAGES: 184-190 CODEN: RJBCET ISSN: 1068-1620 LANGUAGE: English
PUBLISHER: MAIK Nauka/Interperiodica

6/3,K/12 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

133208110 CA: 133(15)208110h JOURNAL
Synthesis of 5'-C- and 2'-O-(bromoalkyl)-substituted ribonucleoside phosphoramidites for the post-synthetic functionalization of oligonucleotides on solid support
AUTHOR(S): Wu, Xiaolin; Pitsch, Stefan
LOCATION: Laboratorium fur Organische Chemie, ETH-Zentrum, CH-8092, Zurich, Switz.
JOURNAL: Helv. Chim. Acta DATE: 2000 VOLUME: 83 NUMBER: 6 PAGES: 1127-1144 CODEN: HCACAV ISSN: 0018-019X LANGUAGE: English PUBLISHER: Verlag Helvetica Chimica Acta
?

```
Set     Items   Description
S1          0   CPG (S) OLIGONUCLEOTIDE? (S) 3' (S) (MODIF? OR SUBSTITUT?)
S2         35   CPG (S) (MODIF? OR SUBSTITUT?) (S) (HALO? OR ALKOXY OR ALK-
              YL)
S3         19   RD (unique items)
S4          0   SHOW FILES
S5         16   S2 AND OLIGO?
S6         12   RD (unique items)
S7          8   S2 AND IMMUN?
S8          3   RD (unique items)
>>>KWIC option is not available in file(s): 399
```

8/3,K/1 (Item 1 from file: 5)

13920501 BIOSIS NO.: 200200549322

Design, synthesis, and immunostimulatory properties of *CpG* DNAs containing *alkyl*-linker *substitutions*: Role of nucleosides in the flanking sequences.

AUTHOR: Yu Dong; Kandimalla Ekambar R; Cong Yanping; Tang Jimmy; Tang Jin-Yan; Zhao Qiuyan; Agrawal Sudhir(a)
AUTHOR ADDRESS: (a)Hybridon, Inc., 345 Vassar Street, Cambridge, MA, 02139 **USA E-Mail: sagrawal@hybridon.com

JOURNAL: Journal of Medicinal Chemistry 45 (20):p4540-4548 September 26, 2002
MEDIUM: print
ISSN: 0022-2623
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

Design, synthesis, and immunostimulatory properties of *CpG* DNAs containing *alkyl*-linker *substitutions*: Role of nucleosides in the flanking sequences.

ABSTRACT: Bacterial and synthetic DNA containing unmethylated *CpG* dinucleotides activate the innate *immune* system and promote Th1-like *immune* responses. Recently, a receptor, TLR9, has been shown to recognize *CpG* DNA and activate *immune* cascade. But there have been no reports on the molecular mechanisms of recognition between *CpG* DNA and the receptor(s). Our earlier studies described a number of the chemical and structural characteristics of *CpG* dinucleotide and the sequences flanking the *CpG* dinucleotide that are critical for immunostimulatory activity. In the present study, we examined the effect of the presence and absence of a nucleoside in the flanking sequences by replacing one or two natural deoxyribonucleosides at various positions with one or more *alkyl*- (C2-C12), branched *alkyl*- (glyceryl or aminobutyryl-propanediol), or ethyleneglycol- (tri or hexa) linkers. The results suggest that a linker *substitution* at the first two nucleoside positions adjacent to the *CpG* dinucleotide on the 5'- or the 3'-side neutralizes the immunostimulatory activity, as determined by in vitro mouse spleen cell proliferation, cytokine secretion, and in vivo mouse spleen enlargement. The same *substitutions* placed about three to six nucleotides away from the *CpG* dinucleotide either did not affect or potentiated immunostimulatory activity compared with parent *CpG*-DNA without *modifications*. *Substitution* of deoxyribonucleosides with a C3 or C4 *alkyl*-linker was found to be optimal for potentiating immunostimulatory activity.

DESCRIPTORS:
...MAJOR CONCEPTS: *Immune* System (Chemical Coordination and Homeostasis
...ORGANISMS: PARTS ETC: blood and lymphatics, *immune* system...

...blood and lymphatics, *immune* system
MISCELLANEOUS TERMS: ...innate *immune* response

DIALOG(R)File 135:NewsRx Weekly Reports

0000071892 (USE FORMAT 7 OR 9 FOR FULLTEXT)
Enhanced *immunomodulatory* activity described
Biotech Week, December 25, 2002, p.63

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English
RECORD TYPE: FULLTEXT
WORD COUNT: 487

Enhanced *immunomodulatory* activity described

TEXT: Hybridon, Inc., (HYBN.OB) announced the publication of a new report describing the enhanced *immunomodulatory* activity of CpG DNA molecules linked through their 3' ends (*immunomers*), as demonstrated in cell culture and murine models.

The report provides insights into designing potent *immunomodulatory* oligonucleotides (IMO) for specific *immunotherapeutic* applications. Hybridon recently described in other publications that linking *CpG* DNA through the 5' end abrogates *immunostimulatory* activity, and that adding *alkyl*-linkers in *substitution* of certain nucleotides in the 5' flanking region - but not the 3' flanking region - increases *immunostimulatory* activity, also as demonstrated in cell culture or murine models.

... the new publication further confirms the previously reported data and begins to elucidate the molecular basis for CpG DNA recognition by receptors that results in *immune* stimulation. These data also highlights the sequence and structural changes in CpG DNA that may potentiate or neutralize *immunostimulatory* activities in a predictable fashion.

"We continue to add building blocks to our understanding of the structure/activity relationship and the design of our proprietary *immunomodulatory* oligonucleotides," said Sudhir Agrawal, Hybridon's chief scientific officer. "We believe that this knowledge will allow us to design and develop potent molecules with specific *immune* responses for the treatment of a broad range of disease conditions."

"This new publication underscores the leading edge work in the field of *immunostimulation* currently underway in Hybridon's labs," stated Stephen R. Seiler, Hybridon's CEO. "As more data is presented by the scientific community validating the ability of CpG DNA molecules to stimulate the *immune* system, Hybridon intends to remain in the forefront of understanding the mechanism of action and designing proprietary compounds that harness the full power of *immunomodulation*. By combining our understanding with a strong intellectual property estate, we believe Hybridon will be the preferred partner in this field."

The study, published in the journal Nucleic Acids Research and entitled "'*Immunomers*' - novel 3'-3' - linked CpG oligodeoxyribonucleotides as potent *immunomodulatory* agents," reports that, in cell cultures and murine models, CpG DNA molecules, when linked through their 3' ends, showed enhanced activity as compared with the...

...molecules, suggesting that the presence of accessible 5' ends is necessary to activate the relevant signaling pathways. The study further demonstrates that the differences in *immunostimulatory* activity of 3' - 3' - and 5' - 5' - linked CpG DNAs do not arise from differences in nuclease stability or their cellular uptake, but from the accessibility of their 5' end(s) for receptor recognition and/or binding.

The studies also examined the optimal length of *immunomers*, and the use of parallel synthesis as a viable manufacturing method. The use of parallel synthesis resulted in higher yields of the *immunomer* products with improved purity.

This article was prepared by Biotech Week editors from staff and other reports.

DESCRIPTORS: Hybridon, Inc.; DNA Research; *Immunology*; Therapy; All News; Consumer News; Biotechweek

0000069544 (USE FORMAT 7 OR 9 FOR FULLTEXT)

Interaction of novel DNA structures and *immune* system activation studied

Biotech Week, November 13, 2002, p.54

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English
RECORD TYPE: FULLTEXT
WORD COUNT: 380

Interaction of novel DNA structures and *immune* system activation studied

...TEXT: of two articles presenting the results of murine cell culture experiments conducted by Hybridon involving the relationships between novel DNA structures and activation of the *immune* system.

The articles, recently published in the Journal of Medicinal Chemistry and in Bioconjugate Chemistry , provide insights into designing potent *immunomodulatory* oligonucleotides (IMOs) for specific *immunotherapeutic* applications, the company says.

... technology platform now being used by an increasing number of companies, we are working to turn the first-generation CpG DNA into specific and potent *immunotherapeutic* agents by incorporating appropriate chemical modifications," said Sudhir Agrawal, D. Phil, Hybridon. "The murine cell culture experiments reported are just a few examples of the on-going effort at Hybridon to develop a thorough understanding of the structure/activity relationships and to create an inventory of proprietary *immunomodulatory* oligonucleotide compounds to effectively treat patients."

The publication in the Journal of Medicinal Chemistry (Design, synthesis, and *immunostimulatory* properties of *CpG* DNAs containing *alkyl*-linker *substitutions*: Role of nucleosides in the flanking sequences. J Med Chem, 2002;45(20):4540-4548), reports that in murine cell culture experiments the *substitution* of certain nucleotides with *alkyl* -linkers in the 5'-flanking sequence to the *CpG* dinucleotide increased *immunostimulatory* activity, whereas the same *substitution* on the 3'-flanking sequence did not affect activity compared with the parent *CpG* DNA. In this study, the increase in *immunostimulatory* activity resulted from the structural *modifications* introduced in the *CpG* DNA.

The Bioconjugate Chemistry (Conjugation of ligands at the 5'-end of CpG DNA affects *immunostimulatory* activity. Bioconjug Chem, 2002;13(5):966-974) publication reports that in murine cell culture experiments the differences in *immunostimulatory* activity of 3'-3'- and 5'-5'-linked DNAs did not arise from differences in nuclease stability or their cellular uptake, but from the accessibility...

...size and nature of the ligand attached to the 5'-end determined the accessibility of that end to receptors in the relevant pathway and the *immunostimulatory* activity. These results potentially have an impact on the studies of CpG DNA-antigen/vaccine/monoclonal antibody conjugates.

This article was prepared by Biotech Week...

DESCRIPTORS: DNA Research; Cell Biology; *Immunology*; All News; Consumer News; Biotechweek

```
Set     Items   Description
S1          0   CPG (S) OLIGONUCLEOTIDE? (S) 3' (S) (MODIF? OR SUBSTITUT?)
S2         35   CPG (S) (MODIF? OR SUBSTITUT?) (S) (HALO? OR ALKOXY OR ALK-
               YL)
S3         19   RD (unique items)
S4          0   SHOW FILES
S5         16   S2 AND OLIGO?
S6         12   RD (unique items)
S7          8   S2 AND IMMUN?
S8          3   RD (unique items)
S9          0   (OLIGONUCLEOTIDE? OR POLYNUCLEOTIDE?) (S) (3' (4N) (SUBSTI-
               TUT? OR MODIF?))
S10      1905   (OLIGONUCLEOTIDE? OR POLYNUCLEOTIDE?) AND (3 (4N) MODIF?)
S11       842   (OLIGONUCLEOTIDE? OR POLYNUCLEOTIDE?) AND (3 (4N) SUBSTITU-
               T?)
S12       161   (S19 OR S10) AND (ALKYL? OR HALO? OR ALKOXY?)
S13       106   RD (unique items)
S14         2   S13 AND (?CG? OR ?CPG?)
S15        14   S13 AND IMMUN?
>>>KWIC option is not available in file(s): 399
```

15/3,K/1 (Item 1 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

03605338 Genuine Article#: PQ557 No. References: 31

Title: SYNTHESIS AND ANTI-HIV ACTIVITY OF *ALKYL* STEROIDAL 3'-AZIDO-3'-DEOXYTHYMIDIN-5'-YL PHOSPHOTRIESTERS AS PRODRUGS OF AZT

Author(s): BALAGOPALA MI; OLLAPALLY AP; LEE HJ
Corporate Source: FLORIDA A&M UNIV,COLL PHARM & PHARMACEUT SCI/TALLAHASSEE//FL/32307; FLORIDA A&M UNIV,COLL PHARM & PHARMACEUT SCI/TALLAHASSEE//FL/32307; FLORIDA A&M UNIV,DEPT CHEM/TALLAHASSEE//FL/32307

Journal: NUCLEOSIDES AND NUCLEOTIDES, 1994, V13, N9, P1843-1853
ISSN: 0732-8311
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

Title: SYNTHESIS AND ANTI-HIV ACTIVITY OF *ALKYL* STEROIDAL 3'-AZIDO-3'-DEOXYTHYMIDIN-5'-YL PHOSPHOTRIESTERS AS PRODRUGS OF AZT

Abstract: *Alkyl* steroidal AZT 5'-monophosphate triesters are designed as lipophilic prodrugs of AZT to improve its therapeutic efficiency. We have synthesized four phosphotriesters of AZT, in...

...Identifiers--NUCLEOTIDE CHEMISTRY; AIDS; DERIVATIVES; 2',3'-DIDEOXYCYTIDINE; *IMMUNODEFICIENCY; *AZIDOTHYMIDINE; INFECTIVITY

Research Fronts: 92-1716 003 (HUMAN-*IMMUNODEFICIENCY*-VIRUS TYPE-1 REVERSE-TRANSCRIPTASE; NONNUCLEOSIDE INHIBITORS; ZIDOVUDINE RESISTANCE; HIV CHEMOTHERAPY; 2',3'-DIDEOXYCYTIDINE INVITRO)

92-1888 001 (HUMAN-*IMMUNODEFICIENCY*-VIRUS TYPE-1; HIV-2 INFECTION; EXTERNAL ENVELOPE GLYCOPROTEIN)

92-2057 001 (OKINAWAN MARINE SPONGE THEONELLA-SWINHOEI; FULLY SATURATED CARBON SKELETON OF LAURENCIA NON-TERPENOID ETHER METABOLITES; LOBOPHYTUM SOFT CORAL)

92-2113 001 (DNA CLEAVAGE; ACTIVE-SITE TYROSINE; RAPID DEPROTECTION OF SYNTHETIC *OLIGONUCLEOTIDES*)

92-2798 001 (ZIDOVUDINE PHARMACOKINETICS; ORAL 3'-AZIDO-*3*'-DEOXYTHYMIDINE PREVENTS SIV INFECTION; *MODIFIED* NUCLEOSIDES SUPPRESSING HUMAN-*IMMUNODEFICIENCY*-VIRUS)

92-5990 001 (HUMAN-*IMMUNODEFICIENCY*-VIRUS TYPE-1; PNEUMOCYSTIS-CARINII PNEUMONIA; AIDS UPDATE; HIV-INFECTED INDIVIDUALS; ORAL INFECTIONS; HOMOSEXUAL MEN)

15/3,K/2 (Item 2 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

03098572 Genuine Article#: NE783 No. References: 50

Title: EFFECT OF DERIVATIZATION OF RIBOPHOSPHATE BACKBONE AND TERMINAL RIBOPHOSPHATE GROUPS IN OLIGORIBONUCLEOTIDES AN THEIR STABILITY AND INTERACTION WITH EUKARYOTIC CELLS

Author(s): BOUTORINE AS; VENYAMINOVA AG; REPKOVA MN; SERGUEYEVA ZA; PYSHNYI DV
Corporate Source: MUSEUM NATL HIST NAT,BIOPHYS LAB,43 RUE CUVIER/F-75231 PARIS 05//FRANCE/; RUSSIAN ACAD SCI,INST BIOORGAN CHEM/NOVOSIBIRSK630090//RUSSIA/
Journal: BIOCHIMIE, 1994, V76, N1, P23-32
ISSN: 0300-9084
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

...Abstract: bonds throughout the molecule, replacement of the two last 3'-terminal phosphodiester bonds by phosphoroamidates and coupling of the last 3'-terminal nucleotide via the *3*'-*3*'-phosphodiester bond. All *modifications* were tested for their effect on the stability of the derivatives against phosphodiesterase from snake venom and nucleases of the cell culture media. 2'-O...

...the corresponding oligodeoxyribonucleotides. 85% of the bound derivatives were found in the membrane-cytosolic fraction, while only 15% were found in the nuclear fraction. The *oligonucleotide* moiety of 2'-O-methyloligoribonucleotide-cholesterol conjugate was not translocated through the cellular membrane. After cleavage of the linkage between cholesterol and *oligonucleotide* by dithiothreitol the major portion of the *oligonucleotide* moiety was released into the media. The derivatives, as well as their 5'-cholesterol conjugates, which entered the cells, were stable and protected from action...
...Identifiers--*ALKYLATING* *OLIGONUCLEOTIDE* DERIVATIVES; ANTISENSE *OLIGONUCLEOTIDES*; NUCLEOTIDE CHEMISTRY; CATALYTIC ACTIVITY; CHEMICAL SYNTHESIS; RNA; DNA; DEGRADATION; PHOSPHATE; ANALOGS
Research Fronts: 92-0799 002 (ANTIBODY ENGINEERING; ANTIGEN COMBINING SITE; FILAMENTOUS PHAGE; PROTEIN TARGETS)
92-0340 001 (ANTISENSE *OLIGONUCLEOTIDES*; HUMAN-*IMMUNODEFICIENCY* -VIRUS TYPE-1 REVERSE TRANSCRIPTION; RIBONUCLEASE H-MEDIATED INHIBITION)

15/3,K/3 (Item 1 from file: 98)

DIALOG(R)File 98:General Sci Abs/Full-Text

04501759 H.W. WILSON RECORD NUMBER: BGSA01001759 (USE FORMAT 7 FOR FULLTEXT)

Evolutionary origin of feathers.

AUGMENTED TITLE: symposium
American Zoologist (Am Zool) v. 40 no4 (Sept. 2000) p. 455-706
ISSN: 0003-1569
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 158028

(USE FORMAT 7 FOR FULLTEXT)

TEXT:

... J. Ornithol. 75:86-223.

Schweitzer, M. H., J. A. Watt, R. Avci, L. Knapp, L. Chiappe, M. Norell, and M. Marshall. 1999. Betakeratin specific *immunological* reactivity in feather-like structures of the Cretaceous alvarezsaurid, Shuvuuia deserti. J. Exp. Zool. 285:146-157.

Sengel, P., D. Dhouailly, and A. Mauger. 1980...explanations for the origin of feathers and associated features as the wing and flight is the rather easy trap of arguing that a particular evolutionary *modification* is "easier" or "simpler" than others, and hence would be the correct explanation. Consider the evolution of the feathered avian wing. In all other gliding...essentially feathered theropod dinosaurs.

The first announced was Sinosauropteryx (Ji and Ji, 1996; Chen et al.,

1998; Ji et al., 1998), which preserves a halo *of* filamentous structures, the longest of which are 30 mm long. They are feather-like in that they are hollow and "resemble most closely the plumules...not preserved in perfect anatomical orientation, but is rotated to the animal's right by a few degrees. The bedding plane--and hence the halo *of* fibers we see--intersects the left dorsolateral surface of the skull. We agree that testing these structures for the presence of keratin and absence of... Science 276:1543-1546.

Schweitzer, M. H., J. A. Watt, R. Avci, L. Knapp, L. Chiappe, M. Norell, and M. Marshall. 1999. Betakeratin specific immunological *reactivity* in feather-like structures of the Cretaceous alvarezsaurid, Shuvuuia deserti. Jour. Exp. Zool. (Mol. Dev. Evol.) 285:146-157.

...9)--another sauropsid synapomorphy.

The classical literature often states that epidermal generations characterize sauropsidan scales (Lange, 1931; Maderson et al., 1998, p. 19). TEM/immunocytochemical *studies* of avian scutae/scutellae show that all suprabasal cells have a-keratin precursors before b-keratins appear (Sawyer et al., 2000). Whatever the adult phenotype...the major structural proteins of feathers. The occurrence of b keratin proteins in the scales and claws of both birds and reptiles and their immunological *cross* -reactivity suggest that the genes for reptilian b keratins may be homologous with those of birds. In bird appendages, the b keratins are the products...

...keratins, from different orders of birds, demonstrate that there is more diversity at the DNA level than was implied by earlier protein sequencing studies.

Immunological *techniques* show that the same antibodies that react with the epidermal b keratins of the chicken (Gallus domesticus) react with the epidermal b keratins of American...relationships between scale morphogenesis and appendage-specific b keratin gene expression are unknown for the alligator. Now, developmental studies of the American alligator, using immunological *and* biochemical approaches, indicate that even though the American alligator does not form epidermal placode and interplacode cell populations during scale formation, it does expresses b keratin polypeptides that are homologous with the avian b keratins.

BIOCHEMICAL AND IMMUNOLOGICAL *CHARACTERIZATION* OF SCALES AND FEATHERS

In 1972, Kemp and Rogers demonstrated that the reduced and S-carboxymethylated (SCM) proteins of scales differ from those of feathers ...

...Carver and Sawyer, 1987), the shell and claws of turtles (Carver, 1988), and the epidermal b keratins of the American alligator (Mays, 1998). Using immunoelectron *microscopic* methods, Shames et al. (1988; 1989) have demonstrated that the anti-b keratin antisera recognize the bundles of 3 nm b keratin filaments in scutate...

...genes are highly conserved (Gregg and Rogers, 1986; Gregg et al., 1983, 1984). Again, Shames et al. (1988) used hybrid selection (with a synthetic oligonucleotide *probe* to the conserved 3' region) and in vitro translation to identify seven scale b keratin polypeptides. All of these polypeptides reacted with the anti-b...proteins (HRPs) (Barnes, 1993; Barnes and Sawyer, 1995) also known as the Fast Proteins (Powell and Rogers, 1979).

INDIVIDUAL SCUTATE SCALE b KERATINS AS IMMUNOGENS

To take advantage of the immunological *approach*, the seven scale b keratin polypeptides, and other polypeptides (Barnes, 1993; Barnes and Sawyer, 1995; Knapp et al., 1991; 1993), were isolated from two-dimensional gels and prepared as immunogens *for* use in rabbits and mice (unpublished data, LWK, RBS and RHS; Shames et al., 1988, 1991). Unlike the S-carboxymethylated polypeptides used as immunogens *by* O'Guin et al. (1982), the epidermal polypeptides, used as immunogens *by* Shames et al (1988; 1989), were extracted by the Triton-X 100/1.5 M KCL method of Franke et al. (1979) and separated by...

...polyclonal antisera for the individual b keratin polypeptides.

THE ANTI-b 1 ANTISERUM
The individual scale beta keratins (b 1-7) were used as immunogens *to* generate polyclonal antibodies in rabbits (unpublished data, LWK, RBS, and RHS). Thus far, characterization of these antibodies indicates that they all react with most of...in the common lizard, Anolis carolinensis. J. Exp. Zool. 243:435-443.

Carver, W. E. and R. H. Sawyer 1988. Avian scale development: XI. Immunoelectron *microscopic* localization of a and b keratins in the scutate scale. J. Morphol. 195:31-43.

Carver, W. E. and R. H. Sawyer 1989. Immunocytochemical *localization* and biochemical analysis of a and b keratins in the avian lingual epithelium. Am. J. Anat. 184:66-75.

Carver, W. E., L. W. Knapp...
...A comparison of genomic coding sequences for feather and scale keratins: Structural and evolutionary implications. EMBO J. 3:175-178.

Haake, A. R. 1985. Immunohistochemical *and* biochemical analyses of keratinization in normal and experimentally recombined tissues of the chick integument. Ph.D. Diss., The University of South Carolina, Columbia, South Carolina...

...feathers and scales. Biochemistry 11:969-975.

Knapp, L. W., P. J. Linser, W. E. Carver, and R. H. Sawyer. 1991. Biochemical identification and immunological *localization* of two non-keratin polypeptides associated with the terminal differentiation of avian scale epidermis. Cell Tissue Res. 265:535-545.

Knapp, L. W., R. B...

...1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12:1133-1142.

O'Guin, W. M. 1984. Biochemical and immunological *characterization* of keratinization in the avian integument: Relationships between morphogenetic and biosynthetic differentiation during embryogenesis. Ph.D. Diss., The University of South Carolina, Columbia, South Carolina.

O'Guin, W. M., L. W. Knapp, and R. H. Sawyer. 1982. Biochemical and immunohistochemical *localization* of alpha and beta keratins in avian scutate scale. J. Exp. Zool. 220:371-376.

O'Guin, W. M. and R. H. Sawyer. 1982. Avian...1972). This review will focus on 1) the unique sebokeratinocytes that make up the avian epidermis; 2) epidermal lipid secretion and permeability barrier formation 3) *the* modifications *of* integumentary structures and their influence on epidermal lipogenesis, and 4) the possible functions of epidermal lipids in birds.

Recent studies on avian epidermal differentiation show...their host by using the fatty acids as chemical cues or host signals. The role of epidermal lipids in host-parasite interactions and potential immune *modulation* is an area of great research interest.

THE INVERSE RELATION BETWEEN FEATHERING AND EPIDERMAL LIPOGENESIS
As is evident from the forgoing account, higher lipogenic potential...

15/3,K/4 (Item 2 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

04050503 H.W. WILSON RECORD NUMBER: BGSA99050503 (USE FORMAT 7 FOR FULLTEXT)
Transformation of leukocytes by Theileria parva and T. annulata.
AUGMENTED TITLE: review
Dobbelaere, Dirk
Heussler, Volker

Annual Review of Microbiology v. 53 (1999) p. 1-42
SPECIAL FEATURES: bibl il ISSN: 0066-4227
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 20954

(USE FORMAT 7 FOR FULLTEXT)

TEXT:
... types for infection were restricted to infected cell lines and parasitized cells isolated from infected cattle. The absence of reactivity with reagents specific for bovine *immunoglobulins* (52, 62) and the fact that a number of parasitized cell lines reacted with a cell surface determinant that was restricted to a subpopulation of...appears to be required for sporozoite entry (152, 153).

Despite the many similarities between T. parva and T. annulata, they infect different cells of the *immune* system. Initial studies revealed that all T. annulata-infected lines were positive for MHC class II, although the amount of class II antigen expressed varied between lines. In addition, all lines were negative for a macrophage/monocyte marker, for surface *immunoglobulin* M (IgM), and the bovine T-cell markers boCD4 or boCD8 (163). In comparative infection experiments with cell populations purified by fluorescent-activated cell sorting...lymphocytolysis occurs, affecting uninfected as well as infected cells, resulting in a marked depletion of lymphoid tissues.

Theileria-infected cells form tumors when injected into *immunocompromised* SCID (70), irradiated (95), or nude mice (96). In athymic nude mice, parasitized bovine cells became widely disseminated in the host's tissues and organs...

...transformed cells induce potent proliferative responses in autologous PBMC (82, 132); this phenomenon is called the autologous Theileria mixed-lymphocyte reaction. PBMC from naive and *immune* animals respond with equal magnitude, suggesting the involvement of a nonspecific, mitogenic component (82).

Research on T. annulata revealed a similar picture. Infection of macrophages...

...cell activity (81) may play an important role in the pathogenesis of theileriosis and contribute to the failure of the host to mount an effective *immune* response in vivo (33).

Theileria thus shares the capacity to induce unspecific T-cell proliferation with a number of viruses, among them Epstein-Barr virus...to be launched for theilericidal drugs, using large-scale in vitro screening. Another important milestone in Theileria research, with important consequences for our understanding of *immune* responses and cell biology, was the discovery that lymphocytes could also be infected in vitro with sporozoites isolated from infected ticks (22). Combined with fluorescent...

...insert). The nature of the interaction between the parasite surface and the host cell cytoskeleton is still unknown. Parasite surface proteins, such as PIM (polymorphic *immunodominant* molecule) or QP protein (15, 177), which are expressed on the surface of schizonts, often contain repetitive elements; such proteins may constitute likely candidates for... protection against the parasite, however, because infected cells flourished during peak IFNg production and only very small amounts of IFNg were produced during the effective *immune* response in *immune* animals. In subsequent work (30), it was shown that the production of IFNg in vivo appears to be tightly controlled by the parasite, in that...

...that uninfected PBMC of cattle with tropical theileriosis or East Coast fever synthesized NO spontaneously in vitro (183a). No was also induced when PBMC of *immune*, but not of native, cattle were cultured with T. annulata-infected cell lines. These results point to NO, produced by macrophages, as a possible mediator of anti-T. annulata activity that might contribute the protective *immune* mechanisms. NO has also been implicated

in vasodilation and, if produced in excess, may induce cell and tissue damage. Therefore, in addition to contributing to...In addition, it was shown that thymidine incorporation of T. annulata-infected cells could be inhibited by 50[percent] in the presence of antisense CK2 *oligonucleotides*, suggesting that proliferation was dependent on functional CK2 (157).

A T. parva CK2 catalytic a-subunit has been cloned (129). The predicted amino acid contains...other cell lines and demonstrated constitutive JNK activity in a number of T. parva- and T. annulata-transformed B cell-and macrophage-derived cell lines. *Immunoblot* analysis with antibodies specific for phosphorylated c-Jun confirmed that activated c-Jun translocated to the nucleus of parasitized, but not of cured cells (19...evolutionarily conserved, coordinating element in the response to stress, injury, and infection. General aspects of NF-kB relating to its function in the regulation of *immune* responses have been reviewed extensively elsewhere (10, 13, 78). NF-kB is a dimer, which consists of members of the Rel protein family. Each family...by the antioxidant N-acetyl-cysteine (NAC) (130) or a range of other antioxidants (J Machado & D Dobbelaere, unpublished information). On the other hand, the *alkylating* agent a-tosyl-phenylalanyl-chloromethyl-ketone, which is widely used as a serine protease inhibitor and which inhibits IkBa phosphorylation, completely inhibited NF-kB translocation...

...stage, one can only speculate about possible mechanisms. In normal cells, NF-kB can be activated by a wealth of different signaling pathways involved in *immune* function and development (149). These encompass pathways involved in innate *immune* responses, including the cytokines TNFa, IL-1a, the chemotactic peptide fMet-Leu-Phe, and the recently identified homologue of Drosophila Toll. Pathways triggered in adaptive *immune* responses include those emanating from T- or B-cell antigen receptors, as well as signals delivered through CD2, CD28 (in the case of T cells...environments, as may be the case for non-lymphoid tissues. These observations raise the possibility that IL-10, derived from parasitized cells, may influence the *immune* responses of naive cattle to infection and that IL-10 expression by Theileria-transformed cells could also contribute to the pathology of theileriosis.

NF-kB...G Langsley, unpublished result). These observations strongly suggest that signals potentially emanating from a PI3-K--linked surface receptor are required for continuous growth.

THE *IMMUNOSUPPRESSANTS* FK506 AND RAPAMYCIN

The macrolide antibiotics FK506 and rapamycin are potent *immunosuppressants* that find application in the field of organ transplanation. Extensive use of these compounds has been made in studies on signal transduction pathways that control...the PI3-K pathway suggest that PI3-K--dependent signaling is crucial for continuous proliferation.

LITERATURE CITED

1. Abraham RT. 1998. Mammalian target of rapamycin: *immunosuppressive* drugs uncover a novel pathway of cytokine receptor signaling. Curr. Opin. *Immunol*. 10:330-36

2. Adamson RE, Hall FR. 1996. Matrix met-alloproteinases mediate the metastatic phenotype of Theileria annulata-transformed cells. Parasitology 113:449-55...

...D. 1996. NF-kB: ten years after. Cell 87:13-20

10. Baeuerle PA, Henkel T. 1994. Function and activation of NF-kB in the *immune* system. Annu. Rev. *Immunol*. 12:141-79

11. Baichwal VR, Baeuerle PA. 1997. Activate NF-kB or die? Curr. Biol. 7:R94-96

12. Baldwin CL, Black SJ, Brown WC, Conrad PA, Goddeeris BM, et al. 1988. Bovine T cells, B cells, and null cells are transformed by the protozoan parasite Theileria parva. Infect. *Immun*. 56:462-67

13. Baldwin CL, Malu MN, Kinuthia SW, Conrad PA, Grootenhuis JG. 1986. Comparative analysis of infection and transformation of lymphocytes from African buffalo and Boran cattle with Theileria parva subsp. parva and T.

parva subsp. lawrencei. Infect. *Immun*. 53:186-91
14. Baldwin CL, Teale AJ. 1987. Alloreactive T cell clones transformed by Theileria parva retain cytolytic activity and antigen specificity. Eur. J. *Immunol*. 17:1859-62
15. Baylis HA, Allsopp BA, Hall R, Carrington M. 1993. Characterisation of a glutamine-and proline-rich protein (QP protein) from Theileria...

...Minn AJ, Noel PJ, June CH, Accavitti MA, et al. 1995. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. *Immunity* 3:87-98
19. Botteron C, Dobbelaere D. 1998. AP-1 and ATF-2 are constitutively activated via the JNK pathway in Theileria parva-transformed...

...Babbage JW, Burgering BM, Groner B, Reif K, Cantrell DA. 1997. Phosphatidylinositol 3-kinase couples the interleukin-2 receptor to the cell cycle regulator E2F. *Immunity* 7:679-89
21. Browder TM, Abrams JS, Wong PM, Nienhuis AW. 1989. Mechanism of autocrine stimulation in hematopoietic cells producing interleukin-3 after retrovirus...

...Brown DJ, Campbell JD, Russell GC, Hopkins J, Glass EJ. 1995. T cell activation by Theileria annulata-infected macrophages correlates with cytokine production. Clin. Exp. *Immunol*. 102:507-14
24. Brown EJ, Beal PA, Keith CT, Chen J, Shin TB, Schreiber SL. 1995. Control of p70 S6 kinase by kinase activity...

...2532-36
26. Brown WC, Logan KS. 1986. Bovine T-cell clones infected with Theileria parva produce a factor with IL 2-like activity. Parasite *Immunol*. 8:189-92
27. Campbell JD, Brown DJ, Glass EJ, Hall FR, Spooner RL. 1994. Theileria annulata sporozoite targets. Parasite *Immunol*. 16:501-5
28. Campbell JDM, Brown DJ, Nichani AK, Howie SEM, Spooner RL, Glass EJ. 1997. A non-protective T-helper-1 response against the intra-macrophage protozoan Theileria annulata. Clin. Exp. *Immunol*. 108:463-70
29. Campbell JDM, Howie SEM, Odling KA, Glass EJ. 1995. Theileria annulata induces abberrant T cell activation in vitro and in vivo. Clin. Exp. *Immunol*. 99:203-10
30. Campbell JDM, Nichani AK, Brown DJ, Glass EJ, Spooner RL. 1998. A stagespecific, parasite-induced, "window" of in vivo interferon-g...

...Ann. NY Acad. Sci. 849:152-54
31. Campbell JDM, Nichani AK, Brown DJ, Howie SEM, Spooner RL, Glass EJ. 1997. Parasite-mediated steps in *immune* response failure during primary Theileria annulata infection. Trop. Anim. Health Prod. 29:S133-35
32. Campbell JDM, Russell GC, Nelson RE, Spooner RL, Glass EJ...

...bovine CD4+ cells from uninfected donors. Biochem. Soc. Trans. 25:S277
33. Campbell JDM, Spooner RL. 1999. Macrophages behaving badly: infected cells and subversion of *immune* responses to Theileria annulata. Parasitol. Today. 15:10-16
34. Cantrell D. 1996. T cell antigen receptor signal transduction pathways. Annu. Rev. *Immunol*. 14:259-74
35. Chaussepied M, Lallemand D, Moreau M-F, Adamson R, Hall R, Langsley G. 1999. Upregulation of Jun and Fos family members...
...Parasitol. 94:215-26
36. Chaussepied M, Langsley G. 1996. Theileria transformation of bovine leukocytes: a parasite model for the study of lymphopro-proliferation. Res. *Immunol*. 147:127-38
37. Chu ZL, DiDonato JA, Hawiger J, Ballard DW. 1998. The tax oncoprotein of human T-cell leukemia virus type 1 associates...

...CJ. 1996. Bovine g/ TcR+ T lymphocytes are stimulated to proliferate by autologous Theileria annulata-infected cells in the presence of interleukin-2. Scand. J. *Immunol*. 44:444-52
41. Coquerelle TM, Eichhorn M, Magnuson NS, Reeves R, Williams RO,

Dobbelaere DA. 1989. Expression and characterization of the interleukin 2 receptor in Theileria parva-infected bovine lymphocytes. Eur. J. *Immunol*. 19:655-59

42. Coscoy L, Gonzalez Dunia D, Tangy F, Syan S, Brahic M, Ozden S. 1998. Molecular mechanism of tumorigenesis in mice transgenic...

...virus tax gene. Virology 248:332-41

43. DeBenedette M, Snow EC. 1991. Induction and regulation of casein kinase II during B lymphocyte activation. J. *Immunol*. 147:2839-45

44. DeMartini JC, Baldwin CL. 1991. Effects of g interferon, tumor necrosis factor a, and interleukin-2 on infection and proliferation of Theileria parva-infected bovine lymphoblasts and production of interferon by parasitized cells. Infect. *Immun*. 59:4540-46

45. Dobbelaere DAE, Coquerelle TM, Roditi IJ, Eichhorn M, Williams RO. 1988. Theileria parva infection induces autocrine growth of bovine lymphocytes. Proc...

...et al. 1990. Expression of Tac antigen component of bovine interleukin-2 receptor in different leukocyte populations infected with Theileria parva or Theileria annulata. Infect. *Immun*. 58:3847-55

47. Dobbelaere DAE, Roditi IJ, Coquerelle TM, Kelke C, Eichhorn M, Williams RO. 1991. Lymphocytes infected with Theileria parva require both cell-cell contact and growth factor to proliferate. Eur. J. *Immunol*. 21:89-95

48. Dobbelaere DAE, Shapiro SZ, Webster P. 1985. Identification of a surface antigen on Theileria parva sporozoites by monoclonal antibody. Proc. Natl...

...Sci. USA 82:1771-75

49. Dobbelaere DAE, Spooner PR. 1985. Production in ascites fluid of biosynthetically labelled monoclonal antibody to Theileria parva sporozoites. J. *Immunol*. Methods 82:209-14

50. Dobbelaere DAE, Spooner PR, Barry WC, Irvin AD. 1984. Monoclonal antibody neutralizes the sporozoite stage of different Theileria parva stocks. Parasite *Immunol*. 6:361-70

51. Dobbelaere DAE, Webster P, Leitch BL, Voigt WP, Irvin AD. 1985. Theileria parva: expression of a sporozoite surface coat antigen. Exp...

...Duffus WP, Wagner GG, Preston JM. 1978. Initial studies on the properties of a bovine lymphoid cell culture line infected with Theileria parva. Clin. Exp. *Immunol*. 34:347-53

53. Dunbar CE, Browder TM, Abrams JS, Nienhuis AW. 1989. COOH-terminal-*modified* interleukin-*3* is retained intracellularly and stimulates autocrine growth. Science 245:1493-96

54. Duyao MP, Buckler AJ, Sonenshein GE. 1990. Interaction of an NF-kB-like...

...216-27

56. Eichhorn M, Dobbelaere D. 1995. Partial inhibition of Theileria parva-infected T-cell proliferation by antisense IL2R a-chain RNA expression. Res. *Immunol*. 146:89-99

57. Eichhorn M, Dobbelaere DAE. 1994. Induction of signal transduction pathways in lymphocytes infected by Theileria parva. Parasit. Today 10:469-72...

...NS, Reeves R, Williams RO, Dobbelaere DAE. 1990. IL-2 can enhance the cyclosporin A-mediated inhibition of Theileria parva-infected T cell proliferation. J. *Immunol*. 144:691-98

59. Eichhorn M, Prospero TD, Heussler VT, Dobbelaere DA. 1993. Antibodies against major histocompatibility complex class II antigens directly inhibit the growth...63. Emery DL, MacHugh ND, Morrison WI. 1988. Theileria parva (Muguga) infects bovine T-lymphocytes in vivo and induces coexpression of BoT4 and BoT8. Parasite *Immunol*. 10:379-91

64. Entrican G, McInnes CJ, Logan M, Preston PM, Martinod S, Brown CG. 1991. Production of interferons by bovine and ovine cell lines infected with Theileria annulata or Theileria parva. Parasite *Immunol*. 13:339-43

65. Farrell PJ. 1998. Signal transduction from the Epstein-Barr virus LMP-1 transforming protein. Trends Microbiol. 6:175-77

66. Fawcett...

...Int. J. Parasitol. 23:77-87

70. Fell AH, Preston PM, Ansell JD. 1990. Establishment of Theileria-infected bovine cell lines in SCID mice. Parasite *Immunol*. 12:335-39

71. Fich C, Klauenberg U, Fleischer B, Broker BM. 1998. Modulation of enzymatic activity of Src-family kinases in bovine T cells...

...cellular kinases. Mol. Cell Biol. 18:5157-65

78. Ghosh S, May MJ, Kopp EB. 1998. NF-kB and Rel proteins: evolutionarily conserved mediators of *immune* responses. Annu. Rev. *Immunol*. 16:225-60

79. Gilmore TD, Koedood M, Piffat KA, White DW. 1996. Rel/NF-kB/IkB proteins and cancer. Oncogene 13:1367-78

80. Glass EJ, Innes EA, Spooner RL, Brown CG. 1989. Infection of bovine monocyte/macrophage populations with Theileria annulata and Theileria parva. Vet. *Immunol*. *Immunopathol*. 22:355-68

81. Glass EJ, Spooner RL. 1990. Parasite-accessory cell interactions in theileriosis. Antigen presentation by Theileria annulata-infected macrophages and production of continuously growing antigen-presenting cell lines. Eur. J. *Immunol*. 20:2491-97

82. Goddeeris BM, Morrison WI. 1987. The bovine autologous Theileria mixed leucocyte reaction: influence of monocytes and phenotype of the parasitized stimulator cell on proliferation and parasite specificity. *Immunology* 60:63-69

83. Good L, Sun SC. 1996. Persistent activation of NF-kB/Rel by human T-cell leukemia virus type 1 tax involves...

...SJ, Scheller LF, Marletta MA, Seguin MC, Klotz FW, et al. 1994. Nitric oxide: cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. *Immunol*. Lett. 43:87-94

85. Hall R, Hunt PD, Carrington M, Simmons D, Williamson S, et al. 1992. Mimicry of elastin repetitive motifs by Theileria...

...43

87. Hauf N, Goebel W, Serfling E, Kuhn M. 1994. Listeria monocytogenes infection enhances transcription factor NF-kB in P388D1 macrophage-like cells. Infect. *Immun*. 62:2740-47

88. Heikkila R, Schwab G, Wickstrom E, Loke SL, Pluznik DH, et al. 1987. A c-myc antisense oligodeoxynucleotide inhibits entry into...

...interleukin (IL)2 receptor expressed on Theileria annulata-infected cells comprises a single IL 2-binding protein. Partial characterization of bovine IL2 receptors. Eur. J. *Immunol*. 19:1339-42

90. Heussler VT, Eichhorn M, Reeves R, Magnuson NS, Williams RO, Dobbelaere DA. 1992. Constitutive IL-2 mRNA expression in lymphocytes, infected with the intracellular parasite Theileria parva. J. *Immunol*. 149:562-67

91. Heussler VT, Fernandez PC, Machado J Jr, Botteron C, Dobbelaere DAE. 1999. N-acetylcysteine blocks apoptosis induced by N-a-tosyl...of bovine lymphosarcoma cells and lymphoid cells infected with Theileria parva in athymic (nude) mice. Nature 255:713-14

97. Irvin AD, Morrison WI. 1987. *Immunopathology*, *immunology* and *immunoprophylaxis* of Theileria infection. In *Immune* responsis in parasitic infections: *Immunology*, *Immunopathology* and *Immunoprophylaxis*, ed. EJL Soulsby, III:223-74. Boca Raton FL: CRC

98. Irvin AD, Ocama JG, Spooner PR. 1982. Cycle of bovine lymphoblastoid cells parasitised by...

...kinase b. Proc. Natl. Acad. Sci. USA 95:9319-24

108. MacMicking J, Xie QW, Nathan C. 1997. Nitric oxide and macrophage function. Annu. Rev. *Immunol*. 15:323-50

109. Malmquist WA, Brown CGD. 1974. Establishment of Theileria parva-infected lymphoblastoid cell lines using homologous feeder layers. Res. Vet. Sci. 16...

...114. McKeever DJ, Nyanjui JK, Ballingall KT. 1997. In vitro infection

with Theileria parva is associated with IL10 expression in all bovine lymphocyte lineages. Parasite *Immunol*. 19:319-24

115. Mehlhorn H, Shein E. 1984. The piroplasms: life cycle and sexual stages. Adv. Parasitol. 23:37-103

116. Montaner S, Perona...

...52:248-60

119. Morrison WI, Goddeeris BM, Brown WC, Baldwin CL, Teale AJ. 1989. Theileria parva in cattle: characterization of infected lymphocytes and the *immune* responses they provoke. Vet. *Immunol*. *Immunopathol*. 20:213-37

120. Morrison WI, Lalor PA, Goddeeris BM, Teale AJ. 1986. Theileriosis: antigens and host-parasite interactions. In Parasite Antigens, Towards New Strategies...

...Dekker

121. Morrison WI, MacHugh ND, Lalor PA. 1996. Pathogenicity of Theileria parva is influenced by the host cell type infected by the parasite. Infect. *Immun*. 64:557-62

122. Muganda PM, Fischer A, Bernal RA. 1995. Identification of a casein kinase activity found elevated in human cytomegalovirus transformed cells. Biochem...Hemmings BA, Ward SG. 1997. Ligation of the T cell co-stimulatory receptor CD28 activates the serine-threonine protein kinase protein kinase B. Eur. J. *Immunol*. 27:2495-501

132. Pearson TW, Lundin LB, Dolan TT, Stagg DA. 1979. Cell-mediated *immunity* to Theileria-transformed cell lines. Nature 281:678-80

133. Perona R, Montaner S, Saniger L, Sanchez Perez I, Bravo R, Lacal JC. 1997. Activation...

...Withey KS, Lundin LB, Roelants GE. 1981. Proliferation and lymphocyte stimulatory capacity of Theileria-infected lymphoblastoid cells before and after the elimination of intracellular parasites. *Immunology* 44:51-60

136. Pinder M, Withey KS, Roelants GE. 1981. Theileria parva parasites transform a subpopulation of T lymphocytes. J. *Immunol*. 127:389-90

137. Pinna LA, Meggio F. 1997. Protein kinase CK2 ("casein kinase-2") and its implication in cell division and proliferation. Prog. Cell...

...1992. Cytokines inhibit the development of trophozoite-infected cells of Theileria annulata and Theileria parva but enhance the proliferation of macroschizont-infected cell lines. Parasite *Immunol*. 14:125-41

139. Richardson JO, Forsyth LM, Brown CGD, Preston PM. 1998. Nitric oxide causes the macroschizonts of Theileria annulata to disappear and host ...

...A, Bisogni R, Tassone PF, et al. 1996. IL-10 inhibits nuclear factor-kB/Rel nuclear activity in CD3-stimulated human peripheral T lymphocytes. J. *Immunol*. 156:2119-23

143. Rothwarf DM, Zandi E, Natoli G, Karin M. 1998. IKK-g is an essential regulatory subunit of the IkB kinase complex...

...144. Sager H, Bertoni G, Jungi TW. 1998. Differences between B cell and macrophage transformation by the bovine parasite, Theileria annulata: a clonal approach. J. *Immunol*. 161:335-41

145. Sager H, Brunschwiler C, Jungi TW. 1998. Interferon production by Theileria annulata-transformed cell lines is restricted to the b family. Parasite *Immunol*. 20:175-82

146. Sager H, Davis WC, Dobbelaere DA, Jungi TW. 1997. Macrophage-parasite relationship in theileriosis. Reversible phenotypic and functional differentiation of macrophages...

...P. 1995. Casein kinase II a transgene-induced murine lymphoma: relation to theileriosis in cattle. Science 267:894-97

149. Sha WC. 1998. Regulation of *immune* responses by NF-kB/Rel transcription factors. J. Exp. Med. 187:143-46

150. Shaw MK. 1995. Mobilization of intrasporozoite Ca2+ is essential for Theileria...

...MK, Tilney LG, McKeever DJ. 1993. Tick salivary gland extract and

interleukin-2 stimulation enhance susceptibility of lymphocytes to infection by Theileria parva sporozoites. Infect. *Immun*. 61:1486-95
155. Shaw MK, Tilney LG, Musoke AJ. 1991. The entry of Theileria parva sporozoites into bovine lymphocytes: evidence for MHC class I...
...89:4461-69
162. Spooner RL, Innes EA, Glass EJ, Brown CGD. 1989. Theileria annulata and T. parva infect and transform different bovine mononuclear cells. *Immunology* 66:284-88
163. Spooner RL, Innes EA, Glass EJ, Millar P, Brown CGD. 1988. Bovine mononuclear cell lines transformed by Theileria parva or Theileria annulata express different subpopulation markers. Parasite *Immunol*. 10:619-29
164. Stagg DA, Chasey D, Young AS, Morzaria SP, Dolan TT. 1980. Synchronization of the division of Theileria macroschizonts and their mammalian...10106-11
170. Taga K, Cherney B, Tosato G. 1993. IL-10 inhibits apoptotic cell death in human T cells starved of IL-2. Int. *Immunol*. 5:1599-608
171. Telerman A, Amson RB, Romasco F, Wybran J, Galand P, Mosselmans R. 1987. Internalization of human T lymphocyte receptors. Eur. J. *Immunol* . 17:991-97
172. Theiler A. 1904. Rhodesian Tick fever. Transvaal Agric. J. 2:421-28
173. Thomas SM, Brugge JS. 1997. Cellular functions regulated...

...human B lymphocytes. J. Biol. Chem. 269:7538-43
177. Toye PG, Goddeeris BM, Iams K, Musoke AJ, Morrison WI. 1991. Characterization of a polymorphic *immunodominant* molecule in sporozoites and schizonts of Theileria parva. Parasite *Immunol*. 13:49-62
178. Tsur I, Adler S. 1963. Growth of Theileria annulata schizonts in monolayer tissue cultures. J. Protozool. Suppl. 10:36
179. Tsur...

...Nitric oxide inhibits establishment of macroschizont-infected cell lines and is produced by macrophages of calves undergoing bovine tropical theileriosis or East Coast fever. Parasite *Immunol*. 17:91-102
184. Ward SG, June CH, Olive D. 1996. PI3-kinase: a pivotal pathway in T-cell activation? *Immunol*. Today 17:187-97
185. Webster P, Dobbelaere DA, Fawcett DW. 1985. The entry of sporozoites of Theileria parva into bovine lymphocytes in vitro. *Immunoelectron* microscopic observations. Eur. J. Cell. Biol. 36:157-62
186. Wells CW, McKeever DJ. 1998. The entry and development of Theileria parva sporozoites within bovine...

...CE, Geppert TD. 1996. MEK1 and the extracellular signal-regulated kinases are required for the stimulation of IL-2 gene transcription in T cells. J. *Immunol*. 156:1020-29
188. Wolf SF, Sieburth D, Sypek J. 1994. Interleukin 12: a key modulator of *immune* function. Stem Cells (Dayt) 12:154-68
189. Wu M, Arsura M, Bellas RE, FitzGerald MJ, Lee HY, et al. 1996. Inhibition of c-myc...

15/3,K/5 (Item 3 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03811053 H.W. WILSON RECORD NUMBER: BGSI98061053 (USE FORMAT 7 FOR FULLTEXT)
Aromatic hydrocarbon receptor polymorphism: development of new methods to correlate genotype with phenotype.
Maier, Andrew
Micka, Jana; Miller, Kevin
Environmental Health Perspectives (Environ Health Perspect) v. 106 no7 (July '98) p. 421-6
SPECIAL FEATURES: bibl il ISSN: 0091-6765
LANGUAGE: English
COUNTRY OF PUBLICATION: United States

WORD COUNT: 5967

(USE FORMAT 7 FOR FULLTEXT)

...ABSTRACT: strains, is an important determinant of environmental toxicity. We took advantage of the Ahr polymorphism in C57BL/6 and DBA/2 mice to develop an *oligonucleotide*-hybridization screening approach for the rapid identification of DNA sequence differences between Ahr alleles. *Oligonucleotides* containing single-base changes at polymorphic sites were immobilized on a solid support and hybridized with C57BL/6 or DBA/2 AHR cDNA radiolabeled probes...

...has been no straightforward method to reliably and reproducibly phenotype large numbers of humans for CYP1A1 inducibility or AHR affinity. Screening human AHR cDNAs by *oligonucleotide*-hybridization and yeast two-hybrid methodologies will be invaluable for the rapid and unequivocal determination of changes in DNA sequence and receptor-ligand affinities associated...

TEXT:

Key words: aromatic hydrocarbon (Ah) receptor, mouse Ahr gene polymorphism, *oligonucleotide* hybridization, yeast two-hybrid system, b-naphthoflavone. Environ Health Perspect 106:421-426 (1998). Online 12 June 1998 http://ehpnet1.niehs.nih.gov/docs/1998...

...the genes controlling the molecular mechanisms of the toxic response. For example, a clear genetic component exists in the susceptibility of mouse strains to many *halogenated* aromatic hydrocarbons (HAHs) and nonhalogenated polycyclic aromatic hydrocarbons (PAHs), such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) and benzo(a)pyrene (BaP), respectively...

...toxicity. BaP is a potent carcinogen in animals and a suspected carcinogen in humans (1). Dioxin is associated with a variety of systemic effects, including *immunosuppression*, cleft palate, and tumor promotion in animals, and chloracne, *immunosuppression*, and possibly cancer and heart disease in humans (2,3). There is no instance known so far in which dioxin or BaP toxicity or carcinogenesis...

...has been limited to small groups of individuals and may be too cumbersome for identification of nucleotide changes in large populations. Approaches that rely on *oligonucleotide* hybridization (30), however, may provide a solution to this problem, particularly in light of the recent advances in DNA chip technology (31).

Detection of nucleotide...of AHR activation (34,39-41). In this report, we have used the well-characterized Ahr polymorphism between B6 and D2 mice to optimize an *oligonucleotide*-hybridization screening approach for the identification of Ahr nucleotide changes and to develop a yeast two-hybrid approach for the functional analysis of the corresponding AHR protein.

MATERIALS AND METHODS

Sequencing by hybridization. Twelve sets of four 21-residue *oligonucleotides* (Table 1), for a total of 48 target *oligonucleotides*, were commercially synthesized (Bio-Synthesis, Inc., Lewisville, TX) for hybridization experiments. Each set corresponded to 1 of the 11 sites at which the Ahrb-1 and Ahrd alleles differed, plus 1 control site at which both alleles were identical. The four *oligonucleotides* in a set differed only by the middle 11th residue, which contained A, C, G, or T. For each set, one *oligonucleotide* matched Ahrb-1, another matched Ahrd, and the other two matched neither.

The sequences and theoretical dissociation temperatures of 23 of the 48 *oligonucleotides* are shown in Table 1. Five of the 11 polymorphic sites encode amino acid sequence changes. *Oligonucleotides* were transferred to a Nytran membrane (Schleicher & Schuell, Keene, NH) using a slot-blotting apparatus (Bio-Rad, Richmond, CA). Each *oligonucleotide* (1 mg) was applied to a slot by filtration under vacuum, washed with 0.5 M

sodium phosphate buffer (pH 7.0), and UV cross...

...the equation:
b-galactosidase activity = (1,000 X A578)/ A600 X cell volume (ml) X time (min) .

RESULTS

Detection of single Ahr nucleotide changes by *oligonucleotide* hybridization. To test whether the *oligonucleotide*-hybridization screening approach could be used to identify nucleotide changes reflecting amino acid differences, we hybridized B6, D2, and chimeric AHR cDNA probes to an *oligonucleotide* array containing single-base substitutions at all mouse Ahr polymorphic sites, plus the one control invariant site. Representative hybridization results are shown in Figure 2 for each of the 12 sets of four *oligonucleotides*. The relative hybridization intensities of all the probes were calculated for each *oligonucleotide* array position, and the means for a given nucleotide position are shown adjacent to the corresponding slot. In all cases, hybridization was strongest with the correct *oligonucleotide*, although we detected varying degrees of hybridization to the other three *oligonucleotides*. Hybridization to Ahrb-
...the 12 D2 probes occurred with the highest degree of specificity. Nonspecific binding was less than 19[percent] of maximum for all positions except for *oligonucleotides* at position 2038--to which, in addition to the *oligonucleotide* containing the correct C nucleotide, *oligonucleotides* containing G and A also hybridized, although with lower efficiencies of 0.85 and 0.40, respectively. Of the other two, two prominent bands, one...

...the correct T and the other at G, were observed for nucleotide position 2038. At position 3336, very little hybridization took place, possibly because this *oligonucleotide* also carried a second nucleotide change at position 3330. Notwithstanding, quantitation showed that hybridization to the correct *oligonucleotide*, containing A at this position, was the strongest.

All hybridization experiments shown in Figure 2 were conducted at 56[degree]C, but we found that the dissociation temperatures for each of the 48 *oligonucleotides* differ widely (Table 1). We tested whether the three hybridizations that generated ambiguous results could be resolved by *modifying* the hybridization temperature.

Figure *3* shows the results of hybridizations conducted at three other temperatures for *oligonucleotides* spanning position 2038 hybridized with both B6 and D2 probes and for *oligonucleotides* spanning position 3336 hybridized with the D2 probe. Regardless of the hybridization temperature used, hybridization was strongest for the correct *oligonucleotide*. Ambiguity for the Ahrb-1 consensus *oligonucleotide* at position 2038 was resolved as the hybridization temperature was increased to approach the dissociation temperature. For the D2 cDNA hybridization at this position, two of the nonspecific bands were resolved, but no clear improvement in discrimination for the other two was seen. Hybridization of D2 cDNA to the *oligonucleotides* spanning position 3336 was unambiguous at 46[degree]C, but the overall intensity was greatly decreased as the temperature was increased, reflecting the lower dissociation temperature due to the presence of a second nucleotide change in the consensus *oligonucleotide* at this position. We concluded that the hybridization patterns clearly show that each probe has a strong preference for the *oligonucleotide* with a perfect sequence match and that this *oligonucleotide*-hybridization technology is capable of detecting single-nucleotide changes with a high degree of accuracy.

The AHR is ligand responsive in a yeast two-hybrid...
...were found to exhibit 14- to 24-fold higher EC50 values than AHR proteins containing Ala-375.

DISCUSSION

We have shown in this report that *oligonucleotide* hybridization screening can be effectively used to identify mouse Ahr nucleotide differences with a high degree of success and reliability. From an array of 48

oligonucleotides containing individual base substitutions at 11 polymorphic sites, specific hybridization was evident for 11 of the 12 Ahrb-1 allele sites and 10 of 12 Ahrd allele sites. We have also shown that hybridization to the incorrect *oligonucleotide* for the other three sites occurred--to varying degrees, causing less-than-definitive results--for two reasons: use of suboptimal hybridization temperature and close proximity...

...the power of this method to identify sequence polymorphisms without the need for DNA sequencing. Although this is the first report of the application of *oligonucleotide* hybridization screening of the Ahr gene, this approach has been used successfully to detect polymorphic alleles of several genes, including bS-globin, HLA-A, BRCA1...of individuals. The complete 2,547-nucleotide coding sequence of the human AHR cDNA could be represented in an array of 849 21-mer sequential *oligonucleotides*, each beginning three nucleotides after the previous one. Any polymorphism, detected by absence of hybridization to the test probe, would be present in seven (21/3) sequential *oligonucleotides*. Inclusion of control and test probes labeled with different fluorescent dyes in the same hybridization reaction would provide a rapid and efficient means of detecting...

...might thus be reflected in the well-documented studies about human variation in CYP1A1 inducibility and AHR affinity.

Our results demonstrate the utility of the *oligonucleotide* hybridization screening approach for identification of the mouse Ahr genotype and of a yeast two-hybrid assay for quantification of the AHR phenotype. Used together...

...was supported by a predoctoral fellowship from the Pharmaceuticals Research and Manufacturers of America Foundation.

Received 9 January 1998; accepted 16 March 1998.

Table 1. *Oligonucleotides* for the detection of mouse Ahr polymorphism

Abbreviations: NT, nucleotide position within the cDNA sequence (12); TD, theoretical dissociation temperature of the perfect hybrid calculated from the sequence; AA, amino acid encoded by the corresponding codon at the polymorphic site. *Oligonucleotides* (21-mers) were synthesized for 10 polymorphic sites in the coding region of the mouse Ahr gene, 1 polymorphic site outside the coding region, and...was replaced by each of the other three nucleotides, one of which matches that of the Ahd allele (shown below each sequence); the other two *oligonucleotides* of each set are not shown. At positions 2679, 2687, 3330, and 3336--due to the proximity of two Ahrb-1/Ahrd polymorphic sites--the Ahrd consensus *oligonucleotides* contain an additional nucleotide change.

Table 2. Median effective concentration (EC50) [plus or minus]SD of b-naphthoflavone induction of B6, D2, and chimeric aromatic...

...P, proline; S, serine; N, glutamic acid; M, methionine; R, arginine.

Figure 2. Hybridization of C57BL/6 (B6) and DBA/2 (D2) cDNA probes to *oligonucleotide* arrays spanning all polymorphic sites in the Ahrb-1 and Ahrd alleles. The hybridization temperature was 56[degree]C. The allele, the nucleotide (NT), and...

...to a representative example of a hybridization experiment. Hybridization intensities were quantitated for all probes, and the mean values relative to the maximum for each *oligonucleotide* in a set are shown.

Figure 3. Resolution of hybridization ambiguities by changing the hybridization temperature. *Oligonucleotides* spanning positions 2038 or 3336 were hybridized at 46[degree]C, 54[degree]C, and 60[degree]C to the C57BL/6 (B6) and DBA...

...dioxins. Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1997.

4. Poland A, Knutson JC. 2,3,7,8,-Tetrachlorodibenzo-p-dioxin and related *halogenated* aromatic hydrocarbons: examination of the mechanisms of toxicity. Annu Rev Pharmacol Toxicol 22:517-554 (1982).

5. Swanson HI, Bradfield CA. The AH receptor: genetics...in mice genetically "nonresponsive" for cytochrome P450 1A1 induction: cytosolic Ah receptor, transformation to the nuclear binding state, and induction of

aryl hydrocarbon hydroxylase by *halogenated* and non-*halogenated* aromatic hydrocarbons in embryonic tissues and cells. Mol Pharmacol 40:818-826 (1991).

19. Niwa A, Kumaki K, Nebert DW. Induction of aryl hydrocarbon hydroxylase...

...the principles of nucleic acid hybridization. Crit Rev Biochem Mol Biol 26:227-259 (1991).

31. Southern EM. DNA chips: analyzing sequence by hybridization to *oligonucleotides* on a large scale. Trends Genet 12:110-115 (1996).

32. Matsushita N, Sogawa K, Ema M, Yoshida A, Fujii-Kuriyama Y. A factor binding...

...1995).

42. Conner BJ, Reyes AA, Morin C, Itakura K, Teplitz RL, Wallace RB. Detection of sickle cell bS-globin allele by hybridization with synthetic *oligonucleotides*. Proc Natl Acad Sci USA 80:278-282 (1983).

43. Bugawan TL, Apple R, Erlich HA. A method for typing polymorphism at the HLA-A locus using PCR amplification and immobilized *oligonucleotide* probes. Tissue Antigens 44:137-147 (1994).

44. Strewing JP, Brody LC, Erdos MR, Kase RG, Giambarresi TR, Smith SA, Collins FS, Tucker MA. Detection...

...Genet 57:1-7 (1995).

45. Hacia JG, Brody LC, Chee MS, Fodor SPA, Collins FS. Detection of heterozygous mutations in BRCA1 using high density *oligonucleotide* arrays and two-colour fluorescence analysis. Nature Genet 14:441-447 (1996).

46. Drobyshev A, Mologina N, Shik V, Pobedimskaya D, Yershov G, Mirzabekov A. Sequence analysis by hybridization with *oligonucleotide* microchip: identification of b-thalassemia mutations. Gene 188:45-52 (1997).

...

15/3,K/6 (Item 4 from file: 98)

03796045 H.W. WILSON RECORD NUMBER: BGSI98046045 (USE FORMAT 7 FOR FULLTEXT)

Ribonuclease P: unity and diversity in a tRNA processing ribozyme.

AUGMENTED TITLE: review

Frank, Daniel N
Pace, Norman R

Annual Review of Biochemistry (Annu Rev Biochem) v. 67 ('98) p. 153-80
SPECIAL FEATURES: bibl il ISSN: 0066-4154
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 11214

(USE FORMAT 7 FOR FULLTEXT)

TEXT:

... structure, metalloenzyme, phylogenetic-comparative analysis

INTRODUCTION: PROPERTIES OF RNASE P

Despite their relatively small sizes, transfer-RNAs are subjected to a complex set of posttranscriptional *modifications* during their biosynthesis (*3*, 4). Almost every species of tRNA is transcribed as a precursor with both 5' and 3' terminal extensions. tRNA genes also often contain introns. These...82) and Zarrinkar et al (83) reported that the folding of B. subtilis RNase P RNA (as monitored by resistance to hydroxyl-radical attack or *oligonucleotide* hybridization, respectively) is cooperatively dependent on Mg2+ concentration and that at least three Mg2+ ions contribute to folding.

In contrast to their contribution to ribozyme...function of bacterial

RNase P proteins .

ARCHAEAL HOLOENZYMES
Archaeal examples of the RNase P holoenzyme have been isolated from only two species: Sulfolobus acidocaldarius and *Haloferax* volcanii. The biochemical properties of the two holoenzymes differ substantially, suggesting rather different organizations to the enzymes. The activity of the H. volcanii enzyme, for...metazoan POP1 peptides (133). Despite the differences in POP1 sequence, antibodies raised against hPOP1p recognize a 115-kDa protein in HeLa cell extracts and can *immunoprecipitate* RNase P activity along with human RNase P RNA (133). Thus, hPOP1p, like its yeast counterpart, is likely to be a component of RNase P...

...of the human RNase P has revealed several potential subunits of the holoenzyme. Autoimmune sera that can both deplete RNase P activity from extracts and *immunoprecipitate* RNase P RNA also recognize ca 38-kDa and 30-kDa polypeptides in highly purified preparations of human RNase P (134-136). The genes encoding...

...have been isolated; both differ in sequence from hPOP1p (136). Furthermore, a 40-kDa protein can be cross-linked to human RNase P RNA and *immunoprecipitated* with anti-Th sera (135, 137); its relationship to the product of RPP38 is not known. Several other proteins copurity with the human RNase P...prior to transport of tRNA transcripts from the nucleus to the cytoplasm (147, 148), indicating that RNase P is resident in the nculeus. However, antisense *oligonucleotides* directed against human RNase P RNA, and detected by fluorescent in situ hybridization, stain cytoplasmic and nucleolar compartments of HeLa cells (149, 150). Whether the...

15/3,K/7 (Item 5 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03796043 H.W. WILSON RECORD NUMBER: BGSI98046043 (USE FORMAT 7 FOR FULLTEXT)
Modified *oligonucleotides*: synthesis and strategy for users.
AUGMENTED TITLE: review
Verma, Sandeep
Eckstein, Fritz
Annual Review of Biochemistry (Annu Rev Biochem) v. 67 ('98) p. 99-134
SPECIAL FEATURES: bibl il ISSN: 0066-4154
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 14342

(USE FORMAT 7 FOR FULLTEXT)
Modified *oligonucleotides*: synthesis and strategy for users.

ABSTRACT: Synthetic *oligonucleotide* analogs have greatly aided our understanding of several biochemical processes. Efficient solid-phase and enzyme-assisted synthetic methods and the availability of modified base analogs have added to the utility of such *oligonucleotides*. In this review, we discuss the applications of synthetic *oligonucleotides* that contain backbone, base, and sugar modifications to investigate the mechanism and stereochemical aspects of biochemical reactions. We also discuss interference mapping of nucleic acid-protein interactions; spectroscopic analysis of biochemical reactions and nucleic acid structures; and nucleic acid cross-linking studies. The automation of *oligonucleotide* synthesis, the development of versatile phosphoramidite reagents, and efficient scale-up have expanded the application of modified *oligonucleotides* to diverse areas of fundamental and applied biological research. Numerous reports have covered *oligonucleotides* for which modifications have been made of the phosphodiester backbone, of the purine and pyrimidine heterocyclic bases, and of the sugar moiety; these modifications serve as structural and mechanistic probes. In this chapter, we review the range, scope, and practical utility of such chemically

modified *oligonucleotides*. Because of space limitations, we discuss only those *oligonucleotides* that contain phosphate and phosphate analogs as internucleotidic linkages. With permission, from the Annual Review of Biochemistry Volume 67, 1998, by Annual Reviews Inc. (http...

TEXT:

KEY WORDS: *oligonucleotide* analogs, backbone modifications, base modifications, sugar modifications, reporter groups

SYNTHETIC METHODS

SOLID-PHASE CHEMICAL SYNTHESIS

The ease of customizing reaction cycles in automated, solid-phase...

...phosphoramidites of suitably protected natural and nonnatural nucleosides, such as 2'-substituted-2'deoxy- and 2'-O-methyl nucleosides, terminal nonradioactive reporter groups, and functionalized *alkyl* tethers for postsynthetic modifications, have greatly added to the convenience of solid-phase synthesis. The phosphoramidite approach, developed for the synthesis of *oligonucleotides* containing a phosphodiester backbone, can also be used to synthesize phosphorothioates by replacing the oxidation step with sulfurization (7). The solid-phase synthesis of other modified backbones, such as phosphorodithioates (8) or methylphosphonates (9), however, requires special nucleoside amidites.

The key features of automated solid-phase *oligonucleotide* synthesis include ease of operation, efficiency, and reproducibility. Despite these advantages, this method is difficult to use for the synthesis of long *oligonucleotides* (100 or more nucleotides) in high yields. In such cases, it is more convenient to use the enzymatic ligation approach, in tandem with chemical synthesis, to give an oligomer of desired length.

ENZYMATIC LIGATION

The enzymatic approach to ligate short *oligonucleotides* offers an attractive route for the synthesis of long deoxynucleic and ribonucleic acids. These reactions are catalyzed by DNA or RNA ligases that promote intermolecular ligation of the 5' and 3' termini of *oligonucleotides* through the formation of a phosphodiester bond.

In the case of DNA ligase, two oligodeoxynucleotides, one bearing a 5'-phosphoryl donor group and another with a free 3'-hydroxyl acceptor, constitute the substrate requirement. It is essential that the phosphorylated and the hydroxyl termini-bearing *oligonucleotides* be present either on homo-(DNA:DNA) or hetero-duplexes (DNA:RNA) (10, 11). *Oligonucleotides* bearing terminal fluorescent and chemiluminescent labels can also act as substrates for DNA ligase. Such modified oligomers have been successfully employed to study cystic fibrosis...

...a phosphodiester bond between a 5'-phosphoryl donor and a free 3'-hydroxyl acceptor group of two single-stranded oligoribonucleotides. It accepts a variety of *oligonucleotide* substrates for ligation. With the use of this enzyme, nucleoside analogs can be site-specifically introduced in oligoribonucleotides at the ligation junction (10). The use...

...the ligation site, thus providing single-stranded 5'-phosphoryl and 3'-hydroxyl termini for enzymatic ligation.

ENZYMATIC INCORPORATION OF NONNATURAL NUCLEOTIDES

The enzymatic synthesis of *oligonucleotides* containing modified bases, sugars, or phosphate groups is a viable alternative to chemical synthesis. Enzymatic incorporation of modified nucleoside triphosphates, with T7 or a similar...5'-b-thiotriphosphate (32), or guanosine 5'-g-thiotriphosphate (33-35) for initiation. Applications of terminal phosphorothioates are discussed in later sections.

POSTSYNTHETIC MODIFICATION OF *OLIGONUCLEOTIDES*

Conjugation of *oligonucleotides* to other biological macromolecules, to nonradioactive reporter groups, to spin labels, and to cross-linking agents has necessitated the development of postsynthetic modification procedures.

The chemical strategies for *oligonucleotide* conjugation have been reviewed (36).

The *modification* of either the *3*'- or the 5'-terminus is a convenient method for equipping an *oligonucleotide* with a reactive aminoalkyl or a mercaptoalkyl group. Introduction of these functionalities in an oligomer can be achieved either through use of appropriate commercial phosphoramidite...

...cystamine can be reacted with the 5'-phosphate group in the presence of a water-soluble carbodiimide. This reaction results in the formation of an *oligonucleotide* containing a reactive functional group at the 5'-terminal. The introduction of a 5'-terminal phosphorothioate group that can be used as a nucleophile is easily achieved through use of ATPgS and *polynucleotide* kinase (33). Alternatively, a 3'-terminal phosphorothioate can be introduced through use of 2'-deoxycytidine-5'-phosphate 3'-phosphorothioate (pdCpS) and RNA ligase (16).

Such *oligonucleotide* conjugates have been used extensively for a number of applications, which include cellular delivery of antisense *oligonucleotides*, synthesis of artificial nucleases, and hybridization probes for biological detection. Examples include conjugation of *oligonucleotides* to viral fusogenic peptides (37, 38), staphylococcal nuclease and other enzymes (39, 40), neoglycopeptides (41), phospholipids (42), thiolated polysaccharides (43), vitamin E (44), lipophilic groups...
...feature makes it useful for cell culture and in vivo studies. The literature on nucleoside phosphorothioates up to 1984 has been reviewed by Eckstein (22).

Oligonucleotide phosphorothioates can be synthesized either by chemical synthesis, with use of a sulfurizing reagent in the oxidation step, or by enzymatic incorporation. Chemical synthesis produces...the phosphorothioate groups accessible to iodine cleavage. Conversely, phosphorothioates can also become hyperreactive by a similar mechanism.

NUCLEASE STABILITY Phosphorothioate modification increases nuclease stability of *oligonucleotides*. This fact was first demonstrated in cell culture experiments with interferon-inducing *polynucleotides* (97). This property is particularly important when *oligonucleotides* are employed for in vivo therapeutic purposes such as the antisense *oligonucleotide* -mediated inhibition of gene expression. The applications of antisense phosphorothioate *oligonucleotides* were reviewed recently (98).

Resistance of phosphorothioate-modified *oligonucleotides* to exonuclease III has allowed for the delineation of the borders of the thymine dimer removal by the human nucleotide excision nuclease by filling the...

...than 100-fold for a phosphorothioate substitution when compared to the phosphate group (101). Similarly, introduction of a single 3'-phosphorothioate also inhibits degradation of *oligonucleotide* primers by the 3'-exonuclease activity of the thermostable DNA polymerases Vent and Pfu (102), an important consideration for the use of such primers in polymerase chain reactions.

Nuclease resistance of phosphorothioate internucleotide linkages, particularly to the restriction enzyme NciI, is the basis of an *oligonucleotide*-directed, site-specific mutagenesis procedure (103, 104). Interestingly, to confer complete nuclease resistance, the position 5' to the cleavage site also has to be a...

...act as an efficient nucleophile for the site-specific attachment of reporter groups, such as photoaffinty agents (108) and fluorescent dyes (109), to oligodeoxynucleotides via *alkylation* reactions. The resulting triesters are very stable below pH 7 but are rapidly hydrolyzed under alkaline conditions. However, the rate of hydrolysis is remarkably slow for double-stranded *oligonucleotides* (109). Such triester formation is not possible for phosphorothioate-substituted oligoribonucleotides because the 2'-hydroxyl group readily reacts to form a cyclic 2',3'-phosphate...

...for oligoribonucleotides (74).

MERCURY ION INTERACTION As mentioned before, phosphorothioate

monoesters interact strongly with mercury (II), and this property has been used to purify modified *oligonucleotides* by mercury-affinity techniques. In contrast, phosphorothioate diesters, which are present in the internucleotidic linkage, interact poorly with mercury (II), as evident by the lack...

...thiolated tRNA on mercury (II)-polyacrylamide gels (33). Surprisingly, a recent study demonstrated that a mercury (II) complex could be prepared using a phosphorothioate-containing *oligonucleotide* and g-resolvase. This complex was used further for X-ray structural analysis (110). A similar structural study has been reported for a phosphorothioate oligodeoxynucleotide...

...protein complex (111). However, this method might not be general because nonspecific interactions with protein sulfhydryl groups may cause interference.

TERMINAL PHOSPHOROTHIOATES FOR COUPLING REACTIONS *Oligonucleotides* with a phosphorothioate at the termini can be prepared by chemical synthesis (112, 113), by enzymatic thiophosphorylation at the 5' end with ATPgS (33, 114...

...end of oligoribonucleotides (16), or by initiating transcription with guanosine derivatives such as GMPS (31), GTPbS (32), and GTPgS (33, 35). Terminal phosphorothioate groups in *oligonucleotides* have been used for a variety of purposes, such as (a) the separation of *oligonucleotides* or transcripts on mercury gels or columns (32, 33, 35), (b) reaction with *haloacetyl* derivatives of fluorescent dyes (16) and bromoacetyl agarose for the affinity purification of DNA-binding proteins (115), (c) reaction with photoaffinity agents (31), (d) reaction with intercalators (112), (e) the template-directed coupling of two *oligonucleotides* (113, 116), and (f) cross-linking a 5'-phosphorothioate *oligonucleotide* to its complementary sequence by reacting it with trans-platinum(II)diamine dichloride (114). NH- AND S-BRIDGING PHOSPHATE INTERNUCLEOTIDIC LINKAGES Another series of *oligonucleotides* have been prepared through the replacement of either the 3'- or the 5'-bridging oxygen of the phosphodiester internucleotidic linkage with sulfur or nitrogen. Oligodeoxynucleotides...

...observation is not surprising because sulfur is a poor nucleophile at phosphorus and requires a good leaving group to form the P-S bond (127).

Oligonucleotides with such linkages have also been used to probe the mechanism of ribozyme catalysis. In the group I intron ribozyme-catalyzed transesterification reaction, cleavage of...
...isostructural and isopolar to the natural nucleic acid backbone, and unlike phosphorothioates, they retain the achiral nature of the phosphorus atom.

Stability studies with phosphorodithioate *oligonucleotides* have demonstrated resistance to a variety of nucleases. The effect was most prominent when the dithioate modification was introduced at alternating internucleotide positions (136). However, introduction of phosphorodithioate linkages in an *oligonucleotide* reduces its binding to a complementary target, induces secondary structures in single-stranded oligomers (137), and enhances binding of modified *oligonucleotides* to proteins (138). Despite these potential drawbacks, phosphorodithioate-modified antisense *oligonucleotides* have been shown to interfere with the expression of erbB-2 mRNA associated with breast cancer (139), to inhibit HIV-1 reverse transcriptase activity (140...BASE MODIFICATIONS
The heterocyclic ring of purine and pyrimidine base provides hydrogen-bonding functional groups in nucleic acids. Carefully designed base analogs, when introduced into *oligonucleotides*, can provide information on the importance of specific functional groups in natural bases. In interpreting results obtained with base analogs, even a subtle change in...
...N-7 nitrogen atom, are therefore valuable probes for studying the role of N-7 nitrogens in recognition. These analogs can easily be incorporated

in *oligonucleotides*, either by enzymatic means (153) or by automated solid-phase synthesis (154, 155). Because of altered base stacking, *oligonucleotides* containing 7-deazapurine analogs destabilize DNA duplex slightly, and the effect is sequence dependent (156).

The interaction of the trp repressor with the trp EDCBA...

...7 nitrogens in the folding of G-rich telomere sequences was investigated through the use of 7-deazaadenine and 7-deazaguanine substitutions (162). G-rich *oligonucleotide* sequences possess a high propensity to form tetraplexes through hydrogen bonding between the N-7 nitrogen of one guanine and the N-2 hydrogen of...

...the structure-function relationship of the hammerhead ribozyme (180).

The role of conserved deoxycytidine residues in template recognition by bacteriophage T7 primase was studied with *oligonucleotides* containing 2-pyrimidinone and 5-methyldeoxycytidine bases (181). The importance of the hydrogen-bonding framework for protein-DNA recognition in conjunction with other nucleoside substitutions was demonstrated.

Oligonucleotides containing a propynyl substituent at the C-5 position of pyrimidines possess high binding affinity toward complementary RNA targets and display a gene-specific antisense effect (182, 183). When such modified *oligonucleotides* additionally contain phosphorothioates, they can be used to target low and high copy messages with equal efficiency (184). Remarkably, a sequence-specific inhibition of gene...

...selectivity in recognizing complementary bases. A universal base can be defined as an analog that can substitute for any of the four natural bases in *oligonucleotides* without significantly impairing the duplex stability. In general, universal base analogs use aromatic ring stacking, instead of specific hydrogen bonds, to stabilize a duplex. However, universal recognition by imidazole-4-carboxamide nucleoside has been attributed to specific hydrogen-bonding contacts (186).

The efficiency of *oligonucleotide* primers containing multiple substitutions of 5-nitroindole (187, 188) and 3-nitropyrrole (189, 190) has been studied and compared in DNA sequencing and in polymerase...used at primer sites corresponding to degenerate base positions and specifically where the sequence data is incomplete. The use of imidazole-4-carboxamide nucleoside in *oligonucleotide* templates results in nucleotide misincorporation during PCR amplification, and it has been suggested that this property can be further used to generate mutant gene libraries (186).

Depending on the length of the *oligonucleotides*, a true mismatch in a DNA hybrid is often difficult to detect. A recent study used 3-nitropyrrole-containing *oligonucleotide* probes to analyze either a PCR-amplified or an allele-specific PCR-amplified polymorphic region of the HLA-DRB locus by artificial mismatch hybridization (191...

...role of active-site 2'-hydroxyl groups in group II intron molecular recognition and catalysis (208). Recently, incorporation of 2'-deoxy-2'-mercaptocytidine into an *oligonucleotide*, via suitably protected phosphoramidite, has been reported (209). However, enzymatic incorporation of 2'-mercapto group-containing modified nucleotides remains to be established.

2'-Fluoro- and 2'-difluoro-2'-deoxyuridine--containing *oligonucleotides* have been used to investigate the mechanism of human G/T glycosylase (210). The modified *oligonucleotides* resisted the excision repair activity of the enzyme and resulted in the formation of a tight-binding DNA-glycosylase complex. Based on these observations, a...

...used, nuclease-resistant aptamers were isolated against the basic fibroblast growth factor (212), human thyroid-stimulating hormone (213), a monoclonal antibody that recognizes the main *immunogenic* region of the human ACh receptor (214), and the keratinocyte growth factor (215).

2'-O-methyl-modified, biotinylated oligoribonucleotides have been used as antisense probes...RNase resistance to these ribozyme constructs (221).

The O-4' ring oxygen can also be replaced by sulfur to result in 4'-thiofuranose modification, and *oligonucleotides* containing such modification have been used to probe protein-DNA interactions such as in the EcoRV restriction endonuclease and methylase systems (125, 222). In one ...

...in a 25-mer to study mechanism-based inhibition of an E. coli DNA repair enzyme, 3-methyladenine DNA glycosylase II (224).

NONRADIOACTIVE LABELS OF *OLIGONUCLEOTIDES*

FLUORESCENT LABELS

BASE ANALOGS 2-Aminopurine (2AP), a fluorescent nucleoside analog, has been used extensively to detect changes in *oligonucleotide* conformation (225). It can substitute for adenosine in base pairing with thymidine without distorting the double helix. The absorption and excitation maximum for 2AP is at 330 nm and has an emission at 380 nm. The quantum yield of 2AP fluorescence, when substituted in *oligonucleotides*, depends on the degree of base stacking (226). Therefore, any change in its fluorescence is a sensitive indicator of structural perturbations in the modified *oligonucleotide* and provides an insight into the dynamics of such processes. Temperature-dependent conformational changes in *oligonucleotides* (227) and the dynamics of mismatched base pairs in oligodeoxynucleotides have been followed in the monitoring of change in fluorescence intensity of 2AP (228). Several reports have described the use of 2AP-containing *oligonucleotides* to study base-pairing interactions in DNA polymerase 3'-5'-exonuclease proofreading (229-231), kinetics of nucleotide incorporation with the Klenow fragment and T4 DNA...

...the lack of base-pairing capabilities (237).

Fluorescent reporter groups have also been attached to the 5-position of deoxyuridine, via aminoalkyl linkers, to follow *oligonucleotide* -protein interactions. One such study with template/primer *oligonucleotides*, where a fluorescent dansyl probe was appended at various nucleotide positions from the 3'-end of the primer, indicated that the Klenow polymerase remains in contact with the template/primer up to six nucleotides from the 3'-end (238). Similarly, contacts between a 42-mer *oligonucleotide* and the E. coli regulatory protein TyrR were also identified through the use of such nucleoside analogs (239).

A fluorescent derivative has also been attached...

...reaction of internucleotidic phosphorothioates with 5-iodoacetamido-eosin and -fluorescein (241). Distances calculated from fluorescence resonance energy transfer (FRET) studies that used the fluorescently tagged *oligonucleotides* were in reasonable agreement with B-DNA helical structure.

A variety of reporter groups have been attached sequence-specifically to oligodeoxynucleotides by the oxidation ofand dissociation of the hairpin ribozyme (245). There is considerable literature on fluorescent dyes attached at the termini of *oligonucleotides*, particularly for FRET studies; this literature has been reviewed elsewhere (225).

ELECTRON SPIN RESONANCE PROBES

Electron paramagnetic resonance (EPR) is an elegant method of monitoring global tumbling and internal motion of *oligonucleotides*. A nitroxide spinglobal probe has been attached to the 5-position of deoxyuridine, via a mono- or diacetylene tether, to form a complementary base pair...

...found to be consistent with the distortion of the DNA as seen in the X-ray structure. Such distortion was not observed with a nonsubstrate *oligonucleotide*. These results document the utility of this analog for its use in Raman spectroscopic studies.

NUCLEIC ACID CROSS-LINKING

Chemically modified *oligonucleotides* are extremely useful in fixing conformations by introducing cross-links in DNA, RNA, or nucleic acid-protein complexes. Site-specific cross-linking permits the determination...

...249-251). In this method, uridine and inosine derivatives possessing good leaving groups at the 4 or 6 position, respectively, are incorporated site-specifically in *oligonucleotides*. Subsequent reaction of the convertible nucleoside, at the *oligonucleotide* level, with a symmetrical q, q-dithiobis(*alkylamine*), results in the formation of cytidine and adenosine derivatives bearing an *alkyl* disulfide tether. Reduction of the disulfide linkage results in the formation of a free sulfhydryl group, which can be reoxidized to form a cross-link...

...strand separation in the DNA-(cytosine-5) methyltransferase-catalyzed reaction (253). It was suggested that reduction in the rate of methylation of the cross-linked *oligonucleotide* was due to the extrusion of the cytosine to an extrahelical position in the normal substrate *oligonucleotide*. Oligoribonucleotides can also be cross-linked in this manner, as exemplified by the formation of an intramolecular disulfide in an RNA ministem loop (254).

In...

...the N-3 of a pyrimidine base, such as in N-3-thioethylthymidine (255). When incorporated at the 3'-end and 5'-end of two *oligonucleotides*, the sulfhydryl group can be oxidized, thus linking two *oligonucleotides* through the formation of disulfide loops. The cross-link imparts greater thermal stability and a unique conformation. Mercaptoethyl groups can also be attached to C5...4-thiopyrimidine and 6-thiodeoxyguanosine requires special protection of the sulfhydryl group (267, 270). The EcoRV restriction endonuclease and modification methylase have been investigated with *oligonucleotides* containing these analogs (267). These analogs could be cross-linked to the enzymes with higher yields for 4-thiothymidine compared to 6-thiodeoxyguanosine. Unfortunately, *oligonucleotides* without the recognition sequences were also cross-linked as a result of nonspecific binding.

4-Thiouridine has been incorporated at specific positions in an adenovirus...

...incorporation into DNA or RNA, respectively, for a subsequent streptavidin-affinity isolation (278). Many biotin phosphoramidites are commercially available for solid-phase chemical coupling to *oligonucleotides*. A similar approach uses incorporation of probes such as digoxigenin (DIG) or fluorescein instead of biotin. For photo-cross-linking, azido-nitrobenzoate has been coupled to the 5'-position, via an amino linker, and the nucleotide has been incorporated chemically into *oligonucleotides* to allow study of the contact with the Klenow fragment (279), T4 DNA polymerase (280), and E. coli DNA polymerase III (281).

In one study...transplatin-modified oligoribonucleotides was demonstrated through the introduction of cross-links in Ha-ras mRNA and through concomitant inhibition of cell proliferation.

CONCLUSIONS

Chemically modified *oligonucleotides* have been immensely helpful in understanding mechanistic and stereochemical aspects of numerous biochemical reactions and processes. The imagination and ability of chemists will ensure that...

...3, D-37075 Gottingen, Germany

ACKNOWLEDGMENT

We thank Paul A. Heaton for valuable comments on the manuscript.

Figure 1 Modified nucleotides for transcription.

Figure 2 *Modified* *oligonucleotide* internucleotidic linkages.

Figure *3* Base and sugar *modifications*.

LITERATURE CITED

1. Caruthers MH, Beaton G, Wu JV, Wiesler W. 1992. Methods Enzymol. 211:3-20

2. Beaucage SL. 1993. Methods Mol. Biol. 20:33-61
3. Brown T, Brown DJS. 1991. In *Oligonucleotides* and Analogues. A Practical Approach, ed. F Eckstein, pp. 1-24. Oxford: IRL
4. Wincott F, Direnzo A, Grimm S, Tracz D, Workman C, et...

DESCRIPTORS:
Oligonucleotides--

15/3,K/8 (Item 6 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03546771 H.W. WILSON RECORD NUMBER: BGSI97046771 (USE FORMAT 7 FOR FULLTEXT)
Ribosomes and translation.
Green, Rachel
Noller, Harry F
Annual Review of Biochemistry (Annu Rev Biochem) v. 66 ('97) p. 679-716
SPECIAL FEATURES: bibl il ISSN: 0066-4154
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 18635

(USE FORMAT 7 FOR FULLTEXT)

TEXT:
... for binding puromycin.

A direct test of the role of the E site has been carried out by Wintermeyer and coworkers (10). They showed that *modifications* of the *3*'-end of tRNA that strongly decrease its binding affinity for the E site (but have negligible effect on its affinity for the A and P...been eliminated as crystals of ribosomes and ribosomal subunits have been obtained, and some of these diffract to relatively high resolution. Diffraction of crystals of *Haloarcula* marismortui 50S subunits extends to a resolution of better than 3 A (35, 36), and preliminary attempts to obtain phase information for these crystals, using...proteins: L3, L4, L13, L17, L20, L22, and L23. There is reasonably good, although not complete, agreement between these positions and those derived from immuno-*electron* microscopy methods (68).

MOLECULAR MODELING

In the absence of high-resolution crystal structures, molecular biologists have traditionally turned to molecular modeling. In some cases, most...16S rRNA that are critically involved in A-site tRNA or mRNA binding.

Taking a reductionist approach, Purohit & Stern (106) characterized a 49-nucleotide oligonucleotide *analog* of the decoding region of the 30S subunit encompassing both its P- and A-site regions. In this study, the authors identified footprints of aminoglycoside...

...decoding region. More controversial is the proximity of the 530 region. Though protected by the binding of both A- and P-site-bound tRNAs, immuno-*electron* microscopy evidence places this region on the opposite side of the 30S subunit from the decoding site (108, 109). Extensive cross-linking analysis of 16S...domain IV in the P site remain protected in the absence of the CCA tail of tRNA. Indeed, in the P site, minimal tRNA oligonucleotide *fragments*, such as CAACCA-(F-Met), protect exactly those nucleotides whose protection was lost in the 3'-terminal deletion series, including all but one of the...

...tRNA-Phe bound in both the A and the P site of the ribosome to nucleotide A2439 of 23S rRNA (122). Interestingly, amicetin-resistant Halobacterium *halobium* *ribosomes* carrying a mutation at the neighboring nucleotide position 2438 (E. coli numbering) (123) are hypersensitive to thiostrepton (124), an antibiotic known to interact with domain...

...site-bound tRNA (137). Allele-specific primer extension was used to assess the binding capabilities of ribosomes carrying mutations at G2252. Wild-type tRNA oligonucleotide *fragment* [CACCA-(N-Ac-Phe)] binds strongly to wild-type ribosomes but is unable to bind to ribosomes carrying mutations at G2252. By contrast, tRNA oligonucleotide *fragments* carrying mutations at C74 [e.g. CAACA-(N-Ac-Phe) and CAUCA-(N-Ac-Phe)] bind only to those ribosomes carrying the corresponding Watson-Crick...organism that carries a single copy of its ribosomal RNA genes. Recently, an rRNA operon-based transformation system was described for the archaeon H. halobium (*159*); the identification of antibiotic resistance mutations in the 23S rRNA gene of Sulfolobus acidocaldarius should allow for the development of a similar transformation system for...L2 has essentially no effect on peptidyl transferase activity (194). Wittmann-Liebold et al (195) similarly report that mutations at histidine 229 of H. halobium *L2* result in functionally inactive ribosomes in a heterologous in vivo E. coli expression system. There are, however, several cautionary notes. Given the substantial background (20...

15/3,K/9 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

136000583 CA: 136(1)583u PATENT
Guanosine tetrad-forming anti-viral guanosine-rich oligonucleotides for use in the treatment of HIV infection
INVENTOR(AUTHOR): Rando, Robert F.; Fennewald, Susan; Zendeguik, Joseph G.; Ojwang, Joshua O.; Hogan, Michael E.
LOCATION: USA
ASSIGNEE: United States Dept. of Health and Human Services
PATENT: United States ; US 6323185 B1 DATE: 20011127
APPLICATION: US 682255 (19960717) *US 53027 (19930423) *US 145704 (19931028) *WO 94US4529 (19940425) *US PV1505 (19950719) *US 535168 (19951023) *US PV13688 (19960319) *US PV14007 (19960325) *US PV16271 (19960417) *US PV15714 (19960423)
PAGES: 141 pp., Cont.-in-part of U.S. Ser. No. 535,168. CODEN: USXXAM
LANGUAGE: English CLASS: 514044000; A61K-031/70; C07H-021/00

15/3,K/10 (Item 1 from file: 35)
DIALOG(R)File 35:Dissertation Abs Online

01570501 ORDER NO: AAD97-24007
***OLIGONUCLEOTIDE* THERAPEUTICS FOR CANCER (DNA)**
Author: GRAY, GARY DALE
Degree: PH.D.
Year: 1997
Corporate Source/Institution: UNIVERSITY OF SOUTH FLORIDA (0206)
Source: VOLUME 58/03-B OF DISSERTATION ABSTRACTS INTERNATIONAL. PAGE 1298. 189 PAGES

***OLIGONUCLEOTIDE* THERAPEUTICS FOR CANCER (DNA)**

The goal of the experiments was to examine the potential therapeutic efficacy of rationally designed antisense *oligonucleotides* targeted against oncogenic c-myc or H-ras RNA transcripts. In an initial experiment, tumorigenic NIH-3T3 mouse fibroblasts transformed by the human T24 H...

...susceptible to enzymatic degradation in biological systems, they are not viable candidates for in vivo experiments. Therefore, experiments were initiated to synthesize and test several *modified* *oligonucleotide* analogs: *3*$\sp\prime$ *alkylamino* oligodeoxynucleoside phosphodiesters, oligodeoxynucleoside phosphorothioates, 2$\sp\prime$-O-methyl oligoribonucleotides, oligodeoxynucleoside methylphosphonates, and peptide nucleic acids. Cell culture experiments focusing on cellular uptake indicated that...

...analog was the most effective with respect to this important attribute. In vivo experiments were therefore conducted using phosphorothioate analogs administered to mice, with circulating *oligonucleotide* levels measured using the fluorophore OliGreen$\sp{TM}.$ One set of experiments examined the inhibitory effects of an anti-ras phosphorothioate on tumors elicited in nude mice by injection of human bladder carcinoma cells carrying an endogenous H-ras oncogene. Several doses and modes of *oligonucleotide* administration were tested, including infusion by sc micro-osmotic pump and periodic sc or ip injections over a period of several weeks. Injections (sc) of 200 nmoles three times per week was the most effective regimen, with the anti-ras *oligonucleotide* inhibiting tumor growth by approximately 80%. The control *oligonucleotide* also inhibited tumor growth although to a much lesser extent, suggesting a non-specific effect of the phosphorothioates. A second set of experiments investigated the effects of an anti-myc *oligonucleotide* on the growth of endogenous lymphomas in Eμ-myc transgenic mice. The *oligonucleotide* was effective at preventing the development of lymphomas in a dose dependent manner, an effect which requires an active *immune* system. Subsequent experiments revealed direct *immunostimulatory* effects of the anti-myc *oligonucleotide* which were not mediated by anti-myc antisense activity but depended on specific sequence motifs and structural characteristics of the *oligonucleotide*.

15/3,K/11 (Item 1 from file: 149)

01311383 SUPPLIER NUMBER: 10762092 (USE FORMAT 7 OR 9 FOR FULL TEXT)

A new cofactor in a prokaryotic enzyme: tryptophan tryptophylquinone as the redox prosthetic group in methylamine dehydrogenase.

McIntire, William S.; Wemmer, David E.; Chistoserdov, Andrei; Lidstrom, Mary E.

Science, v252, n5007, p817(8)
May 10,
1991
PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Academic
WORD COUNT: 5333 LINE COUNT: 00495

... oxidase, and methylamine oxidase from the soil bacterium Arthrobacter P1 [2, 3]. The design of PQQ, an orthoquinone, is appropriate for catalyzing the oxidation of *alkylamines* [4], and several research groups have offered circumstantial evidence that stable derivatives of 2,7,9-tricarboxy-PQQ could be released from several of these...MADH was crucial for deciphering the cofactor structure. This information was provided by DNA sequencing of the cloned gene from M. extorquens AM1 [25]. Two *oligonucleotide* probes were synthesized on the basis of the known amino acid sequence of the small, cofactor-containing subunit of MADH from this organism [22]. One...bacteria that might produce it. Once absorbed, this quinone could interact nonspecifically with various blood and tissue proteins, such as bovine serum albumin and human *immunoglobulin* G [37]. However, it is unlikely that binding would explain the near 1:1 PQQ-subunit stoichiometry reported for various enzymes [8]. The reason for...

...would be applicable to these as well. The two simplest mechanisms for amine oxidation by ortho-quinones are presented in Schemes 1 and 2 (R, *alkyl* oraryl group). Benzylamine oxidation by PQQ in solution can occur by either pathway, with the mechanism shown in Scheme 2 (the aminotransferase mechanism) predominating at pH <10 [4]. The *modified* aminotransferase mechanism in Scheme *3* seems to be operating for the topa quinone-coppercontaining bovine plasma amine oxidase [*3*, 38, 39]. The *modification* was required in order to explain all of the available data for the oxidation of 2-phenylethylamine by this oxidase [39].

The identification of TTQ...

01119309 SUPPLIER NUMBER: 04722870 (USE FORMAT 7 OR 9 FOR FULL TEXT)

Mutants of bovine panceatic trypsin inhibitor lacking cysteines 14 and 38 can fold properly.

Marks, Cara Berman; Naderi, Hossein; Kosen, Phyllis Anne; Kuntz, Irwin D.; Anderson, Stephen

Science, v235, p1370(4)
March 13,
1987
PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Academic
WORD COUNT: 2443 LINE COUNT: 00256

... Cys5 or Cys38/Cys5, in addition to the disulfide Cys30/Cys51. Creighton (1) showed that when cysteines 14 and 38 were blocked by reduction and *alkylation*, the modified BPTI could not readily regain its native conformation when refolded in vitro at 25|C. These results suggested that non-native disulfide bonds...

...14 and 38 were components of a highly preferred pathway for BPTI refolding (1, 3).

The above studies were open to the objectionthat the bulky *alkylating* agents used to block cysteines 14 and 38 sterically interfered with refolding. We therefore decided to repeat this experiment with genetically modified BPTIs in which...

...threonine substitutions. The number of cysteines per polypeptide chain was also measured directly. The proteins were fully reduced with dithiothreitol in the presence of urea, *alkylated* with a mixture of iodoacetate and iodoacetamide, and analyzed by gel electrophoresis (5). Four cysteines were found in each mutant protein (Fig. 1). The gel...

...treatments have been used previously to detect BPTI refolding intermediates in which particular disulfide bonds were missing (3, 4). The mutant proteins were resistant to *alkylation* by iodoacetate in the presence of 8M urea (Fig. 2), indicating that no free thiols were present (3). The mutant proteins were also resistant to...

...other criteria the mutant proteinsappeared to have a native-like structure similar to BPTI that had been selectively reduced at the Cys14/Cys38 disulfide and *alkylated* with iodoacetamide ([14-cam,38-cam]BPTI). The mutants migrated with the same mobility on polyacrylamide-urea gels (7) as [14-cam,38-cam]BPTI...
...Misfolded BPTI molecules with incorrect disulfide bonds, as well as molecules with reduced Cys5/Cys55 or Cys30/Cys51 disulfide bonds, are inactive against trypsin when *modified* by *alkylation* (1, *3*, 13). Therefore, the iodoacetamide quenching procedure followed by the activity assay effectively discriminated between native-like molecules, in which the Cys5/ Cys55 and Cys30/Cys51...Fig. 3). At 25|C, however, the mutant BPTIs showed a pronounced lag in their refolding behavior (Fig. 3), similar to that reported for BPTI *alkylated* at cysteines 14 and 38 (1).

Previous refolding studies on BPTI invitro revealed two-disulfide intermediates having non-native disulfide bonds between cysteine 5 and...

...whereas at physiological temperatures or above the mutants refold without a significant lag. Finally, there are no discernible differences in the refolding behavior of BPTI *alkylated* at cysteines 14 and 38 and BPTI in which alanine or threonine have been substituted for these residues.

Our results with BPTI at 37|C...

...for the homologous snake proteins, mamba inhibitors I and K, at 25|C (16). Inhibitors I and K with cysteines 14 and 38 blocked by *alkylation* readily refold at 25|C, albeit at a rate severalfold slower than the rates

for unmodified inhibitors. The refolding of the *alkylated* proteins apparently occurs via the direct formation of the Cys30/Cys51 and Cys5/Cys55 disulfides without the participation of non-native disulfide bonds (16). Temperatures...

...have been observed for bovine trypsinogen when disulfide bridges were removed by chemical modification (19, 20). These results, together with the data for reduced and *alkylated* mamba inhibitors (16), imply that the formation of at least some disulfide bonds can provide a modest driving force for protein folding. There is no...

...BPTI refolding intermediates missing only theCys14/Cys38 disulfide bond appear to have a nativelike conformation (1) and are active in the trypsin inhibition assay when *alkylated* with iodoacetamide (9).

14. T. E. Creighton, J. Mol. Biol. 87, 579 (1974).

15. The protein refolding reaction, under our conditions,exhibits complex sigmoidal kinetics...

...254,4291 (1979).

20. A. Light and T. W. Odorzynski, ibid., p. 9162.

21. Mutant BPTI genes were made by site-directedmutagenesis in vitro with *oligonucleotide* primers on an M13 template [M. Zoller and M. Smith, Methods Enzymol. 100, 468 (1983)]. The sequences (antisense strand) of the primers were: Cys14 Thr...

...affinity chromatography as described (4). However, the vector was modified to substitute the heat-stable enterotoxin II signal sequence [C. H. Lee et al., Infect. *Immun*. 42 264 (...40- 1 aliquots were withdrawn, mixed with 10 1 of 0.5M iodoacetamide, and incubated at 25|C for 60 minutes in the dark to *alkylate* protein thiols. Iodoacetamide incubations in the presence of 8M urea, which should expose buried thiols (3), did not result in further modification of the protein (8), indicating that *alkylation* of all thiols was essentially complete under our conditions.

23. The pH of the refolding buffer was adjusted to 8.7 atroom temperature before BPTI...

...1986; accepted 7 January 1987

Table: 1. Susceptibility of correctly folded[Thr14,Thr38]BPTI to urea and dithiothreitol (DTT). Shown are the expected results for *alkylations* performed as described by Marks et al. (4). Actual results for wild-type and [Thr14,Thr38]BPTI are given in (4) and in Fig. 2...

...were then blocked by adjusting the samples to a final concentration of 50 mM iodoacetate, 50 mM iodoacetamide, or 50 mM mixtures of these two *alkylating* agents in various proportions. *Alkylations* proceeded at 25|C for 60 minutes in the dark. *Alkylated* BPTI molecules containing different charges were then resolved by electrophoresis on a 15% acrylamide--8M urea gel (5, 6). The heterogeneity engendered in the population by the mixed *alkylation* resulted in a "ladder' of the variously charged species on the gel, enabling the number of cysteines per polypeptide to be determined by inspection. Gels were stained in 10% trichloroacetic acid, 10% sulfosalicylic acid, 0.1% Coomassie blue (5). Lanes: IAA, iodoacetate; IAM, iodoacetamide; mixture, two samples from *alkylation* reactions containing various proportions of iodoacetate and iodoacetamide (42 mM IAA 8 mM IAM and 25 mM IAA 25 mM IAM); L, ladder produced by...

...0) containing 1 mM EDTA was incubated in the presence of 8M urea, 10 mM dithiothreitol (DTT), or both, for 30 minutes at 37|C. *Alkylations* were performed at 25|C for 60 minutes in the presence of 50 mM iodoacetate (IAA). Samples from each reaction containing 5 g of protein...of time of refolding at 37|C. Symbols: , wild type; , [Thr14,Thr38]BPTI; , [Ala14,Ala38]BPTI; , BPTI with cysteines 14 and 38 blocked by iodoacetamide *alkylation*. (B and C) Results of similar experiments performed at 52| and 25|C, respectively. For the wild-type refolding experiments, similar results were obtained with...

15/3,K/13 (Item 3 from file: 149)

01056916 SUPPLIER NUMBER: 02879721 (USE FORMAT 7 OR 9 FOR FULL TEXT)

Sequence of the 16S ribosomal RNA from *halobecterium* volcanii, an archaebacterium.

Gupta, Ramesh; Lanter, Jan M.; Woese, Carl R.

Science, v221, p656(4)
August 12,
1983
PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Academic
WORD COUNT: 2055 LINE COUNT: 00213

Sequence of the 16S ribosomal RNA from *halobecterium* volcanii, an archaebacterium.

TEXT:

Sequence of the 16S Ribosomal RNA from *Halobacterium* volcanii, an Archaebacterium

... data for some ribosomal proteins do exist (6).

We now report the sequence of a large ribosomal RNA from an archaebacterium, the 16S rRNA of *Halobacterium* volcanii. In secondary structure, this rRNA resembles the eubacterial 16S rRNA more closely than it resembles the 18S rRNA of eukaryotes. Yet in a number...

...the H. volcanii 16S rRNA aligned with its counterparts from Escherichia coli and Xenopus laevis. The DNA sequence is completely consistent with the catalogs of *oligonucleotides* produced by digestion of the corresponding rRNA by ribonuclease T1 (complete catalog, covering 47 percent of the sequence as pentamers or larger), by pancreatic ribonuclease...than by eubacterial synthetases and that protein synthesis in archaebacteria starts with Met-tRNA, not F-Met-tRNA (23), and (iv) the finding that an *immunological* cross-reaction exists between archae-bacterial and eukaryotic RNA polymerases (24). It is apparent that the matter of the earliest phylogenetic branchings is far from...

...B. E. H. Maden, Nature (London) 291, 205 (1981).

13. Most of these examples are drawn not only from the full sequences, but from rRNA *oligonucleotide* catalogs as well--of which there are more than 200 eubacterial examples, more than 20 archaebacterial examples, and the plant, animal, fungal, and slime mold...Fig. 1. Sequence of the 16S ribosomal RNA from H. volcanii.

The sequence has been determined completely by DNA sequencing (25). Partial RNA sequencing, by *oligonucleotide* cataloging, has determined the 5 and *3* termini, located the *modified* nucleosides, and in part checked the DNA sequence (for accuracy and insertions) (7). Modified residues are shown in lowercase but are not otherwise identified. The...

15/3,K/14 (Item 1 from file: 442)

00087797

Genetic and Infectious Prion Diseases (ARTICLE)

PRUSINER, STANLEY B.
Archives of Neurology
Nov, 1993; Neurological Review: p1129
LINE COUNT: 02660

...nucleic acids./2/ Although it seems likely that transmissible prions are composed only of PrP/Sc/ molecules, a hypothetical second component such as a small *polynucleotide* remains a formal possibility.

Studies on the structure of PrP/Sc/ and PrP/C/ have been unsuccessful in defining a posttranslational chemical modification that distinguishes... 38/ At the same time, tests done in search of a scrapie-specific nucleic acid were unable to demonstrate any dependence of infectivity on a *polynucleotide* ,/8/ in agreement with earlier studies reporting the extreme resistance of infectivity to UV irradiation at 254 nm./39/

Based on these observations, it seemed...

...but as they age neoplasms develop./44/

TERMINOLOGY

The term prion is used to denote the small proteinaceous infectiousparticles that resist inactivation by procedures that *modify* nucleic acids (Table *3*). Prions cause scrapie and other related transmissibleneurodegenerative diseases of animals and humans (Table 1). Prions are composed largely, if not entirely, of a protein designated...and a cheetah. Three cases of FSE in domestic cats have been transmitted tolaboratory mice, and PrP/Sc/ has been identified in their brains by *immunoblotting* ./66/ Spongiform encephalopathies have been found in the brains of five exotic ungulates in British zoos; brain extracts prepared from a nyala and a greater...

...leads to death in a comparatively short time. With a few exceptions, the neuropathologic changes in animals are similar to those described here for man.

Immunologic Studies

The rapid and reliable diagnosis of CJD post mortem can be accomplshed using antisera to PrP./90-93/ Initially, partial purification of 1 to...

... of infected brain tissue using detergent extractions, differential centrifugation, and enzyme digestions was required to detect PrP/Sc/ in 14 cases of CJD analyzed by *immunoblots*./92/ Antibodies raised against SHa PrP 27-30 were found to react with protease-resistant PrP in the partially purified fractions prepared from human CJD...

...and all of these demonstrated the presence of CJD prion proteins. Brains from control patients withanoxic encephalopathy or AD did not contain these protease-resistant,*immunoreactive* prion proteins. Recently, two new procedures have beendeveloped for diagnostic evaluations of CJD; one protocol uses a dot blot where ...most part, replaced transmission studies using apes and monkeys.

In one study, 100% of the CJD cases (14/14) examined were found to have PrP *immunoreactive* proteins that were proteinase K-resistant./92/ In another report, 17 (71%) of 24 CJD cases showed PrP *immunoreactive* proteins by *immunoblotting* with PrP 27-30 antiserum samples./95/ All 24 of those cases had been previously transmitted to apes and monkeys. Since considerable data indicate that...

...the human prion protein.

In support of this argument that all cases of CJD should have demonstrable CJD prion proteins is our experience with the *immunostaining* of CJD amyloid plaques. In 16 (94%) of 17 CJD cases with amyloid plaques we have found PrP *immunoreactive* plaques./46,98,99/ Since all cases of CJD do not have readily identifiable amyloid plaques, *immunostaining* of tissue sections fixedin formaldehyde and embedded in paraffin is only useful when positive. The use of other fixatives such as McLean's appears to...difficulties transmitting human kuru prions to apes and monkeys orally are due to the inefficiency of thisroute/36/ and the crossing of a species barrier.

Immunologic and Molecular Biological Studies

Using PrP antiserum, protease-resistant *immunoreactive* proteins have been demonstrated in the brain extracts of one (50%) of two patients dying of kuru./91/ Presumably, these two patients are included in... diagnostic conclusions can as yet be made from such transmission studies becausetransmissibility of GSS appears to be variable, even within a single pedigree./11,168/

Immunologic Studies

Using PrP antiserum, protease-resistant *immunoreactive* proteins

have been demonstrated in the brain extracts of two (50%) of four patients with GSS./91/ Presumably, these two patients are included ina second...

... of the prion protein./95/ Partially purified protease-resistant prion proteins from the brain of one patient dying of GSS were to found be PrP *immunoreactive* on Western *immunoblots* ./92/ Subsequent studies with larger numbers of GSS patients have documented the PrP *immunoreactivity* of amyloid plaques and the presence of PrP/Sc/./169,170/ A dot blot procedure for detection of PrP/Sc/ in brain homogenates provides a...

... that senile plaques have a collection of amorphous material, presumably degenerating dendrites, around their amyloid core. The kuru plaques do not possess such a large *halo* . Both kuru and senile plaques have been reported in CJD, but they are not a constant feature of the disease./186,187/ Kuru plaquesdo seem... GSS. In addition to the multicentric plaques of GSS, unicentric kuru plaques may also be seen./11/

In numerous cases of GSS, there are PrP-*immunoreactive* proteins within the amyloid plaques./98,99,189/ Thus, like CJD and kuru, the amyloid plaques of GSS specifically stain with antisera raised against PrP 27-30 that was isolated from scrapie-infected hamster brains. The PrP *immunostaining* of formalin-fixed brain tissue embedded in paraffin blocks as well as dot blot *immunostaining* of PrP/Sc/ in brain homogenates can be used to establish the diagnosis of GSS./93,98/

The pattern of white matter degeneration resembles that...

... spongiform changes/11/ until Tateishi and coworkers/190/ demonstrated transmission to rodents froma case of GSS with minimal spongiform changes.

Prior to the availability of *immunocytochemical* analyses for PrP, some cases of GSS were incorrectly diagnosed as familial AD, since histochemistry showed NFTs and amyloid plaques of the Alzheimer type./165 ...

... be incidental, the distribution of NFTs throughout the cerebral cortex along with plaques suggests AD or the simultaneous occurrence of AD and GSS./165/ Subsequent *immunostaining* studies have showed that the amyloid plaques in some of these cases are composed of prion proteinsand do not bind antibodies raised to the B...

... pathologic hallmarks of kuru. Most, but not all, cases of kuru exhibit amyloid plaques./69/ These plaques have been found to contain prion proteins by *immunostaining*./99/

MOLECULAR GENETICS OF INHERITED HUMAN PRION DISEASES

Human PrP Gene Mutations

In humans, genetics were first thought to have a role in CJD with... well as some in other families were once thought tohave familial AD but now are known to have prion diseases on the basis of PrP *immunostaining* of amyloid plaques and PrP gene mutations./192,194,232,233/ Patients with the codon 198 mutation havenumerous NFTs that stain with antibodies to T... caused by a viruslike particle that contains a scrapie-specific nucleic acid that encodes the information expressed by each isolate./264/ To date, no such *polynucleotide* has been identified by a wide variety of techniques including measurements of the nucleic acids in purified preparations.An alternative hypothesis has been suggested where...

... is unknown. Although the search for a scrapie-specific nucleic acid continues to be unrewarding, some investigators steadfastly cling to the notion that this putative *polynucleotide* drives prion replication. If prions are found to contain a scrapie-specific nucleic acid, then such a molecule would be expected to direct scrapie agent...

... using a strategy similar to that employed by viruses (Figure 6, A). In the absence of any chemical or physical evidence for a scrapie-specific *polynucleotide* ,/1,239-249,270-277/ it seems reasonable to consider some alternative mechanisms that might feature in prion biosynthesis.The multiplication of prion infectivity is...linked glycosylation of PrP/Sc/ or variations in PrP/Sc/ conformation is uncertain.

Consider the remote possibility that prions do contain an as yet

undetected *polynucleotide* ; then, presumably, prion replication would involve a viruslike strategy. The putative scrapie-specific nucleic acid would act as a template for its own synthesis using...

... in humans without deleterious effects as is the case for Prn-p/0/0/ mice,/21/ then reducing the level of PrP mRNA with antisense *oligonucleotides* might prove an effective therapeutic maneuver in delaying the onset of CNS symptoms and signs.

The study of prion biology and diseases seems to be...MP, Ryan JBM. Bovine spongiform encephalopathy: epidemiological studies. Vet Rec. 1988;123:638-644.

57.

Wilesmith J, Wells GAH. Bovine spongiform encephalopathy. Curr Top Microbiol *Immunol*. 1991;172:21-38.

58.

Dealler SF, Lacey RW. Transmissible spongiform encephalopathies: the threat of BSE to man. Food Microbiol. 1990;7:253-279.

59...SB. Creutzfeldt-Jakob disease prion proteins in human brains. N Engl J Med. 1985;312:73-78.

91.

Brown P, Coker-Vann M, Gajdusek DC. *Immunological* study of patientswith Creutzfeldt-Jakob disease and other chronic neurological disorders: Western blot recognition of infection-specific proteins byscrapie virus antibody. In: Bignami A, Bolis...

... Nervous System Disorders. Geneva, Switzerland: Foundation for the Study of the Nervous System; 1986:107-109.

92.

Bockman JM, Prusiner SB, Tateishi J, Kingsbury DT. *Immunoblotting* of Creutzfeldt-Jakob disease prion proteins: host species-specific epitopes. Ann Neurol. 1987;21:589-595.

93.

Serban D, Taraboulos A, DeArmond SJ, Prusiner SB...

...551.

96.

Prusiner SB. Prions causing degenerative neurological diseases. Annu Rev Med. 1987;38:381-398.

97.

Gabizon R, McKinley MP, Groth DF, Prusiner SB. *Immunoaffinity* purification and neutralization of scrapie prion infectivity. Proc Natl Acad Sci U S A. 1988;85:6617-6621.

98.

Kitamoto T, Tateishi J, Tashima I...

... Creutzfeldt-Jakob disease stain with prion protein antibodies. Ann Neurol. 1986;20:204-208.

99.

Roberts GW, Lofthouse R, Brown R, et al. Prion-protein *immunoreactivity* in human transmissible dementias. N Engl J Med. 1986;315:1231-1233.

100.

DeArmond SJ, Mobley WC, DeMott DL, et al. Changes in the localization ...infectious agent originate in the human genome? BMJ. 1985;291:299-302.

169.

Tateishi J, Kitamoto T, Hashiguchi H, Shii H. Gerstmann-Straussler-Scheinker disease: *immunohistological* and experimental studies. Ann Neurol. 1988;24:35-40.

170.

Tateishi J, Kitamoto T, Doh-ura K, et al. *Immunochemical* , moleculargenetic, and transmission studies on a case of Gerstmann-Straussler-Scheinker syndrome. Neurology. 1990;40:1578-1581.

171.

Tagliavini F, Prelli F, Ghisto J, et...in rodents. Presented at the Sixth International Congress of Virology, September 1-7, 1984; Sendai, Japan. Page 76.

185.

Kitamoto T, Tateishi J, Sato Y. *Immunohistochemical* verification ofsenile and kuru plaques in Creutzfeldt-Jakob disease and the allied disease. Ann Neurol. 1988;24:537-542.

186.

Chou SM, Martin JD. Kuru...

... N, Allsop D, Glenner GG. A variant of Gerstmann-Straussler-Scheinker disease with B-protein epitopes and dystrophic neurites in the peripheral regions of PrP-*immunoreactive* amyloid plaques. In: Natvig JB, Forre O, Husby G, et al, eds. Amyloidand Amyloidosis. Dordrecht, the Netherlands: Kluwer Academic Publishers; 1991;2:737-740.

196...

?